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Self-organisation and forces in the microtubule cytoskeleton

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Modern microscopy techniques allow us to observe specifically tagged proteins in live cells. We can now see directly that many cellular structures, for example mitotic spindles, are in fact dynamic assemblies. Their apparent stability results from out-of-equilibrium stochastic interactions at the molecular level. Recent studies have shown that the spindles can form even after centrosomes are destroyed, and that they can even form around DNA-coated beads devoid of kinetochores. Moreover, conditions have been produced in which microtubule asters interact even in the absence of chromatin. Together, these observations suggest that the spindle can be experimentally deconstructed, and that its defining characteristics can be studied in a simplified context, in the absence of the full division machinery.

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Introduction

In biology, the term 'self-organisation' can have different meanings. Most commonly, it refers to an organisation process in which multiple agents (e.g. molecules, animals, etc.) follow behavioural rules based on local information. Self-organisation requires the absence of a preconceived vision of the final organisation—a *plan* that would be followed by the agents, or imposed on them by a leader [1].

In physics and chemistry, this definition is not of much use, simply because the systems studied are made of agents that are too simple, of molecules that lack intelligence. Thus, the above definition of 'self-organisation' always applies, and the term is used instead as a synonym of out-of-equilibrium organisation [2,3]. In this more precise sense, a self-organised system continuously *consumes and dissipates* energy to maintain itself. This should be opposed to 'self-assembling' systems, which instead release free energy during their organisation, leading to *static* structures in which no energy flows. Self-assembly and self-organisation thus cover the two basic possibilities

with respect to energy requirements. As will be illustrated later, typical organisation phenomena contain both self-assembling and self-organising parts.

The cytoskeleton as a self-organising system

The cytoskeleton is the basis of the internal architecture of eukaryotic cells, and its organisation seems to emerge mostly from self-organisation. This is already the case for the key components of the cytoskeleton: the polar filaments generated by the non-covalent assembly of tubulin or actin subunits. This assembly is coupled to GTP and ATP hydrolysis, for microtubules and actin, respectively [4,5]. The *dynamic* filaments found in cells are self-organised structures because they persistently consume energy. Microtubules or actin filaments can also be said to be self-assembled structures, because they can be formed even when GTP hydrolysis is inhibited [6] — that is, without sustained consumption of energy. It might be perplexing that both self-assembly and self-organisation can produce microtubules; however, the two types of microtubules are of a different nature. Their tubulin lattices, although similar, look different on electron micrographs [7,8]. This difference would become striking if we could detect the forces and tensions inside the structure. While a static microtubule grown in the presence of a non-hydrolysable GTP analogue is a tube that has little tensions, a dynamic microtubule grown in the presence of GTP is a tube ready to crack. During growth, the energy supplied by GTP hydrolysis is stored in the lattice as mechanical strain. This strain powers the fast shortening of disassembling microtubules [9–11]. This simple example shows how difficult it could be to make the distinction between self-organisation and self-assembly. Forces are not easy to see, and the best way to detect self-organisation is to look at the dynamical properties of the system. Dynamic instability and treadmilling are phenomena that require energy dissipation, and which could not emerge from a pure self-assembly process [12–15].

Thus, tubulin or actin monomers self-organise into dynamic filaments. The next level of complexity emerges from the self-organisation of those filaments into various three-dimensional patterns. This involves other components, such as regulators of filament nucleation and dynamics, as well as molecular motors [16]. In contrast to chemical systems, such as the Belouzov–Zhabotinsky reaction [17,18], mechanical forces are central to the self-organisation of the cytoskeleton, simply because of the size of those filaments. Indeed, while small molecules can diffuse and react rapidly to organise themselves in space,

the diffusion of cytoskeletal filaments is too slow to play a significant role in their organisation over the time scale of the life of a cell. Various mechanisms have evolved to make up for this slow diffusion. One such mechanism is the directional transport of the filaments using, for example, mechanical forces generated by molecular motors that consume energy in the form of ATP hydrolysis [19]. In addition, confinement [20], gravitation, gradients of monomer concentrations and steric interactions between filaments have been shown *in vitro* to contribute to the self-organisation of filaments [21]. Another mechanism is to nucleate the filaments directly at the right place. Other strategies involve the remarkable dynamic properties of filament ends. The spatial regulation of microtubule dynamic instability can also result in the disassembly of the filaments of unwanted characteristics. To understand the morphogenesis of cytoskeletal systems and ultimately of whole cells, we need to determine the relative contribution of nucleation, transport and dynamic stabilisation to the generation of the observed patterns [22]. Here, we will review the relative contribution of those processes to the formation of microtubule asters and mitotic spindles.

Pathways of aster formation

In animal cells, microtubules are often organised in the form of an aster, with their plus ends radiating towards the periphery of the cell and the minus ends focused near the nucleus. One familiar mechanism leading to this particular organisation is based on localising nucleation: tubulin cannot assemble spontaneously in the cytoplasm and the centrosome determines the origin of microtubule growth [23] (Figure 1a). This naturally results in the generation of a radial pattern of uniform polarity. In fact, at least two other pathways exist (Figure 1). One of these pathways was discovered by examining the formation of microtubule asters in *Xenopus* mitotic egg extracts exposed to the microtubule-stabilising drug taxol. In these extracts, taxol induces the random assembly of microtubules throughout the cytoplasm that get reorganised into asters under the action of the motor dynein (Figure 1b) [24]. A third pathway was observed in fragments of fish scale melanophores [25,26] (Figure 1c). Although it was initially believed that these asters also formed under the action of motors binding several filaments, it turned out that the microtubules were not moved [27•], leading the authors to formulate a new hypothesis based on the transport of local nucleators. In these cell fragments, pigment granules appear to nucleate microtubules. Because the granules are coated with dynein, they are transported to microtubule minus ends. This creates a positive feedback for local aggregation of granules and concurrent microtubule nucleation that leads to the formation of asters. In the cell fragment, the competition for granules between many asters finally results in pooling all the granules together. Microtubules soon depolymerise from regions devoid of granules, leaving a single, large aster.

An aster formed off a centrosome is purely the consequence of a precise spatial control of nucleation. The formation of asters by oligomeric motors relies on the directed transport of the filaments by the motors, under uncontrolled nucleating conditions. The aster formation observed in fish scale melanophores is an interesting mixture of transport and nucleation. Whereas aster formation from a centrosome could occur by pure self-assembly (with non-dynamic filaments, however), the two other examples of aster formation are true self-organisation phenomena. These systems remind us of an important lesson that we learned from out-of-equilibrium physics and chemistry: although they involve very few components and simple principles, they can be surprisingly difficult to understand. There is, as yet, no satisfactory analytical theory that can predict the correct outcome of motor-mediated self-organisation from the many kinetic rates describing the interactions of the components. The somewhat easier numerical approaches [28] have been used to verify the intuitive models derived from these experiments and to explore potential pathways that may be hidden in the experimental observations.

We will now examine how self-organisation processes may be involved in a more complex structure: the mitotic spindle. By doing so, we will first discuss the characteristic features of the spindle and then examine how they can be studied separately.

Characteristic features of the spindle

During cell division, all cytoplasmic microtubules usually disappear, to be replaced by a mitotic spindle that is assembled to physically segregate the chromosomes into two equivalent pools [29]. The universal trait essential to this function is bipolarity. This does not necessarily mean having two well-focused poles; but it does mean that the microtubule organisation in the spindle should define two opposite directions along which chromosome segregation can proceed. On a more detailed level, another essential trait present in the spindle is the amphitelic attachment of kinetochores, which is needed to pull the sister chromatids in opposite directions. Furthermore, the forces applied to the kinetochore would tend to collapse the two poles together, if they were not compensated for by opposing forces originating either from a pole–pole physical connection — independent of kinetochore-microtubules and able to withstand compression — or from a connection of the poles to a rigid cell cortex via astral microtubules. The pole–pole connection is usually realised by a central antiparallel overlap of microtubules originating from each pole, which can be observed directly after the onset of anaphase. Secondary traits, which might not be essential to all cells, include having well-focused poles, aligned chromatids, or kinetochores that form a narrow metaphase plate. For instance, while spindles in the animal kingdom have their poles well

Figure 1
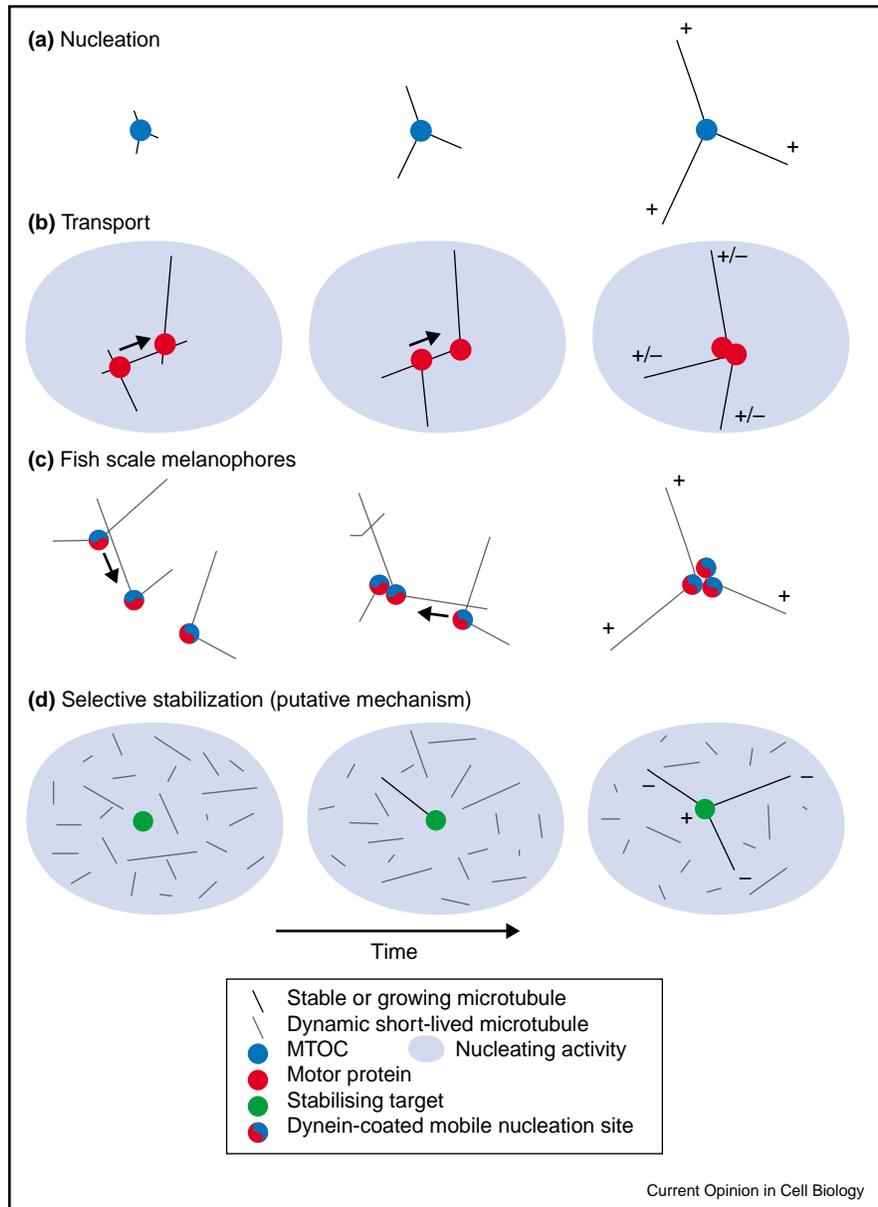
Polar radial arrays of microtubules — asters — can be formed in different ways. **(a)** Nucleation by a microtubule-organising centre, followed by growth at the plus end. **(b)** Nucleation throughout the sample, followed by motor-dependent reorganisation. By moving on two microtubules simultaneously, oligomeric motors organise the microtubules in space. Homo-complexes made of minus-end-directed motors produce asters with normal polarity, while complexes of plus-end-directed motors produce asters of opposite polarity, with the minus ends out. **(c)** Nucleation by pigment granules, which are transported along microtubules by the motor dynein. Granules aggregate at microtubule minus ends, while dynamic microtubules disassemble in regions free of granules. **(d)** Apart from these processes that have been observed in living matter, either under natural or forced conditions, one can imagine other pathways leading to asters. For example, pure selective stabilisation on a single spot would lead to the generation of *inverted* radial arrays with plus ends in the centre. Indeed, let's assume that we create a cytoplasm in which short-lived microtubules are nucleated everywhere and that there are pointed spots of stabilisation distributed in the cytoplasm. All the plus ends that do not hit a stabilising spot will eventually disappear, resulting in the formation of 'inverted asters'.

focused by centrosomes, spindles in the plant kingdom have wider poles [30–32].

The spindle can therefore be described as a combination of simple geometrical characteristics. Most of these traits are somewhat redundant or overlapping, and it might be difficult to isolate them. We should expect, for example, that the focusing of the poles by two centrosomes also imposes bipolarity on the structure. Similarly, an antiparallel overlap of microtubules in the spindle midzone also

creates a nascent bipolarisation of the structure. However, spindle subsystems can also be separated, as shown by experiments carried out on the mitotic spindle in different biological systems. Bipolarity and pole formation can be studied using chromatin-coated beads incubated in mitotic egg extracts in the notable absence of centrosomes and kinetochores [33]. Beads are not segregated, but at least two aspects of spindle assembly can be studied. Using this system, it was possible to show that pole formation in the absence of centrosomes occurs in a process very much similar to the motor-dependent aster formation discussed above. More recent results obtained in living vertebrate cells confirm that a bipolar spindle can form in the absence of centrosomes [34**], according to a pathway that resembles that observed in plant cells.

In contrast, in a growing number of conditions, centrosome-nucleated asters have been observed to interact in the absence of chromatin: in sea urchin [35,36], *Xenopus* lysates [37], in homokaryons generated by fusion of PtK (kangaroo kidney) cells [38*], or in enucleated male spermatocytes [39]. These situations might offer the opportunity to study the antiparallel interactions seen in the spindle independently of the complexity brought by chromatin, a situation that has also been studied theoretically [40**]. In chromosome-free mouse oocytes [41], or in *Xenopus* egg extracts supplemented by a constitutively active mutant form of the regulator protein Ran [42,43], microtubule structures form. Although they lack both chromosomes and centrosomes, these microtubule structures present a bipolar organisation, and possibly also antiparallel interactions — two traits present in the spindle. Even mixtures of purified motors and microtubules can produce self-organised structures showing some characteristics also found in spindles, for example asters or separation of plus- and minus-end-directed motors [44**].

In this review, we focus on a few examples where large pieces of the spindle machinery are missing centrosomes, chromosomes or kinetochores. We do not discuss how specific inhibition of proteins using mutants [45–48], antibody injection or small chemical inhibitors [49] is essential to our understanding of the spindle, and to each of the altered condition cited. Instead, we illustrate how the understanding of the substructures can be extended to that of the mitotic spindle.

Pathways of spindle assembly
Many hypotheses have been proposed to explain the emergence of spindle morphology. For example, the observed capture and selective stabilisation of centrosome-nucleated microtubules by kinetochores [50] was proposed as a general mechanism. This 'search-and-capture' scheme can indeed explain how kinetochores get attached to microtubules in the spindle, but it cannot explain the bipolarity observed in *Xenopus* extracts around

chromatin-coated beads in the absence of centrosomes and kinetochores [33]. To explain these figures, a different view has been proposed in which local microtubule nucleation and stabilisation around chromosomes is coupled to their organisation into a bipolar array by a complex mixture of plus- and minus-end-directed motors [51,52]. This model explains bipolarity, but it cannot explain the formation of specific microtubule attachments to kinetochores, a process essential for chromosome segregation during anaphase.

These two ideas — as well as the experimental systems that provoked them — really address different geometrical traits of the spindle: search-and-capture is an explanation of kinetochore attachments; while motor-mediated self-organisation is mainly an explanation of bipolarity. Only part of the morphology is covered in both cases, and we should therefore combine these ideas (and others) in more complete models, based on the principles of filament organisation (Figure 1). Search-and-capture is a general strategy to connect two spots with microtubules. One spot nucleates in a random direction, while the other spot selectively stabilises microtubules. The model of spindle assembly around chromatin beads is a combination of nucleation and motor-mediated transport. The nucleation is not limited to the small region of the centrosomes but to a halo around chromatin, created by a diffusible gradient of as yet unknown effects [11,53]. Recent results indicate that such a gradient does exist and is locally activated by Ran GTP [54*]. In fact, this gradient may affect both local microtubule nucleation, microtubule dynamics at their plus ends, and motor activities [55*,56*]. In any case, the models are built of the same three principles, but with different spatial regulations, spots or gradients (Figure 2). We can thus foresee a unifying theoretical framework, which could sustain, for example, computer simulations, allowing us to explore more systematically all the formal possibilities offered by different spatial regulations of transport, nucleation and stabilisation.

We should expect explaining spindle morphology to be a formidable challenge. Each of the geometrical characteristics of the spindle (bipolarity, focused poles, antiparallel overlaps, etc.) might be a complicated problem. What we learned from the study of aster formation suggests that a given pattern might be reached through different pathways of self-organisation. Combining such pathways might allow a large number of formal possibilities to form spindle-like shapes.

Meanwhile, a given shape does not warrant a given function. Many of these combinations will probably lead to structures looking like a spindle but unable to segregate chromosomes. Their structure might be incompatible with anaphase; for example, they might be unable to produce sufficient pulling forces on the kinetochores to

Figure 2
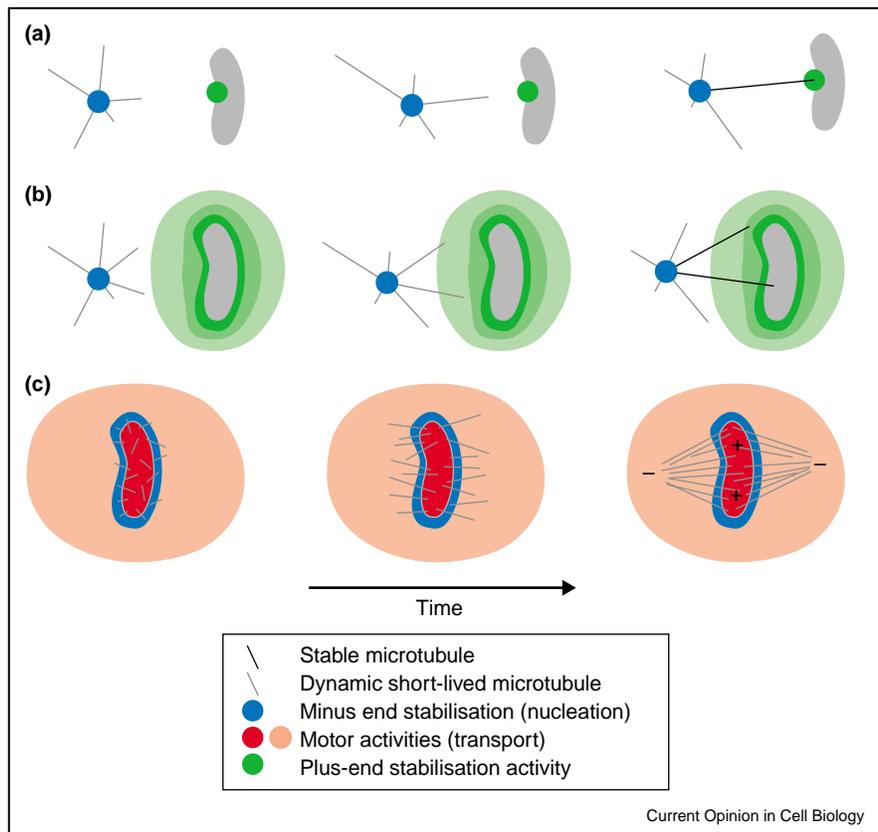
Proposed models of spindle morphogenesis are combinations of nucleation, motor activity and microtubule stabilisation. **(a)** Search-and-capture combines localised nucleation with localised stabilisation. **(b)** Chromosomes could stabilise microtubules a-distance, through an as yet unidentified diffusible factor. **(c)** Chromosomes can also induce the nucleation of microtubules in their vicinity. Together with molecular motors, they would organise into a focused bipolar array.

separate them. In fact, the generation of these forces requires the spindle to be a tensile structure when it reaches a steady state at metaphase. The existence of such tensions has been shown recently by laser micro-surgery in *Caenorhabditis elegans* embryos [57••]. In these experiments, severing the middle of the spindle results in a separation of the two halves that is much faster than the separation of chromosomes observed in anaphase of uncut spindles. Although this technique does not permit mea-surement of the forces quantitatively, the result seems to indicate that the spindle is under much greater tension than that needed to just move the chromosomes apart. These tensions are thought to result from the antagonistic action of a high number of motor proteins [58••,59], whose chemical cycles produce forces and are thus also sensitive to forces in the structure, which can slow them down. Microtubule growth can also sense resisting forces [60]. More surprisingly, tensions at kinetochores are also used for signalling — that is, at the source of a cascade of regulation reactions, resulting in the release of the mitotic check point — allowing mitosis to proceed into anaphase [61••]. Therefore, the morphogenesis of the mitotic spin-

dle is a remarkable self-organisation process, which, on top of creating a very precise shape, also creates its own internal tensions to pull on kinetochores.

Conclusions

Microtubules are organised in various patterns in cells. During interphase, they form radial networks in fibro-blasts and most highly motile cells. In epithelial cells, they are organised as vertical bundles aligned along the apico–basal axis of the cells, while in plant cells they are perpendicular to the main growth axis. During muscle cell differentiation, they form transient large bundles that probably play an important role in myofibrille for-mation [62]. We begin to understand the simplest self-organising systems such as aster formation. These systems teach us that we will have to develop a new perspective on how to study intracellular morphogenesis. In addition to the identification of the key players and key interactions in a given process, it is of highest importance to characterise their dynamics. This will allow us to confront ideas with experimental results, using predictive modelling.

It will be important to understand precisely the spatial modulation of at least three contributions to filaments order: nucleation, stabilisation and transport. We will also need to make a stronger distinction between the 'spatial configuration' and the 'functional configuration' of cytoskeletal structures, taking into account the fact that tensions can be essential to their function. We know already three ways of making such simple shapes as asters. At this stage, one can only wonder how many different pathways can possibly lead, for example, to a spindle-like structure. It will be interesting to see how nature can use and combine different mechanisms in diverse organisms or cell types. In fact, this suggests a strategy that could be followed to unravel the dynamic complexity of self-organised biological systems.

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