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Microtubule self-organisation by reaction-diffusion processes causes collective transport and organisation of cellular particles

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Abstract

Background: The transport of intra-cellular particles by microtubules is a major biological function. Under appropriate *in vitro* conditions, microtubule preparations behave as a 'complex' system and show 'emergent' phenomena. In particular, they form dissipative structures that self-organise over macroscopic distances by a combination of reaction and diffusion.

Results: Here, we show that self-organisation also gives rise to a collective transport of colloidal particles along a specific direction. Particles, such as polystyrene beads, chromosomes, nuclei, and vesicles are carried at speeds of several microns per minute. The process also results in the macroscopic self-organisation of these particles. After self-organisation is completed, they show the same pattern of organisation as the microtubules. Numerical simulations of a population of growing and shrinking microtubules, incorporating experimentally realistic reaction dynamics, predict self-organisation. They forecast that during self-organisation, macroscopic parallel arrays of oriented microtubules form which cross the reaction space in successive waves. Such travelling waves are capable of transporting colloidal particles. The fact that in the simulations, the aligned arrays move along the same direction and at the same speed as the particles move, suggest that this process forms the underlying mechanism for the observed transport properties.

Conclusions: This process constitutes a novel physical chemical mechanism by which chemical energy is converted into collective transport of colloidal particles along a given direction. Self-organisation of this type provides a new mechanism by which intra cellular particles such as chromosomes and vesicles can be displaced and simultaneously organised by microtubules. It is plausible that processes of this type occur *in vivo*.

Background

The collective transport of intracellular particles along a specific cellular direction is a fundamental process in cell biology [1]. In many cases particles move along the direction of orientation of microtubules at speeds of several microns per minute. For example, during cell division, chromosomes travel along the microtubules of the mitotic spindle, and in neuronal axons vesicles move in the direction of orientation of microtubule arrays.

Microtubules [1,2], which also organise the cytosol, are tubular shaped supra-molecular assemblies with inner

and outer diameters of 16 nm and 24 nm. They are often several μm long and arise from the self-assembly of tubulin. *In vitro*, microtubules are formed by warming a solution containing purified tubulin in the presence of guanosine triphosphate (GTP), from about 7°C to 35°C. A series of chemical reactions occurs in which GTP is hydrolysed to guanosine diphosphate, GDP, and within a few minutes, the tubulin assembles into microtubules. This reaction then continues by processes in which the complex, tubulin-GTP, is added to the growing end of a microtubule and the complex tubulin-GDP is lost from the opposite shrinking end, thus causing the microtubule to change position. Excess GTP in the solution then reconverts the liberated tubulin-GDP to tubulin-GTP.

It has been proposed [3-6] that in specific types of chemical reaction which are sufficiently far-from-equilibrium, macroscopic self-organisation might arise from a non-linear coupling of reactive processes with molecular diffusion. As self-organisation requires a continual flux or dissipation of chemical energy through the system, such reaction-diffusion or Turing-type [5] structures are also called dissipative structures. Self-organisation of this type is an example of an 'emergent' phenomenon in a 'complex ' system [7-9]. A particular feature of some 'complex' systems, is that self-organisation is strongly affected by weak external factors that break the symmetry of the self-organising process and so modify the collective behaviour [7-10]. Striped arrangements often arise; when they do, they are nearly always the result of an outside external perturbation that induces a directional bias on the actions of the individual.

Under appropriate conditions the formation *in vitro* of microtubules, shows this type of behaviour [11-18]. Preparations spontaneously self-organise by way of reaction and diffusion, and the morphology that develops depends upon the presence of a weak external factor at a critical time early in the process. Following assembly of an initially homogenous solution of tubulin and GTP into microtubules, a series of stationary periodic horizontal stripes of about 0.5 mm separation progressively develop in the sample over about 5 hours. In each striped band, the microtubules are highly oriented at either 45° or 135°, and adjacent stripes differ in having opposing orientations. This pattern of changes in microtubule orientation coincides with an identical concentration pattern; the microtubule concentration drops by about 25% and then rises again every time the microtubule orientation flips from acute to obtuse. Within each individual band, there is another series of stripes of about 100 μm separation. These bands, in their turn, contain a further set of stripes of about 20 μm separation. At distances below this, there are other levels of organisation of about 5 μm and 1 μm separation. Self-organisation also arises when samples are prepared in small containers of dimensions (50–200 μm) comparable to cells and embryos.

Various experiments show that self-organisation is not a result of static interactions such as give rise to lyotropic liquid crystalline phases, but comes about by reactive processes associated with microtubule formation and maintenance [11-18]. Numerical simulations based on the chemical dynamics of a population of growing and shrinking microtubules successfully predict the main features of the experimental behaviour [16,17]. They provide insight as to how self-organisation occurs at a microscopic level by way of the microtubule reaction dynamics and how weak external factors trigger this process.

We thought it possible that microtubule self-organisation by reaction and diffusion might also result in the transport of intracellular particles. Here, we report experiments that show that this is the case. Self-organisation gives rise to a collective transport of micron sized colloidal particles, such as, polystyrene beads, vesicles, nuclei, and chromosomes, along the same direction. The particles move, along the direction of microtubule orientation, at speeds of several microns per minute. No further movement occurs once self-organisation is completed. In addition, after self-organisation, the colloidal particles show the same pattern of organisation as the microtubules. To examine how this behaviour comes about at a microscopic level, we have carried numerical simulations using the algorithm already described [16,17]. We find that the simulations predict the formation during self-organisation of micron sized arrays of oriented microtubules. In each array, all the microtubules collectively advance along the same direction in a front. Other parallel arrays follow each advancing front of growing and shrinking microtubules. These fronts move across the reaction space in the same direction and at a speed of several microns per minute. Since these travelling waves involve differences in both concentration and viscosity, they can also transport colloidal particles. The fact that in the simulations, the aligned arrays move along the same direction and at the same speed as the particles move, suggests that this process forms the underlying mechanism for the observed transport properties.

Results and discussion

Experimental observations

Microtubules were formed by warming preparations of purified tubulin (10 mg ml^{-1}) in the presence of GTP (2 mM) in a suitable buffer, from 7 to 35°C, on the hot stage of a microscope. The preparations, contained in glass spectrophotometer cells measuring 4 cm by 1 cm by 1 mm, also contained colloidal particles of polystyrene beads, 1.1 μm in diameter. For some preparations, the fluorophore, 4',6-diamidino-2-phenylindole (DAPI), was

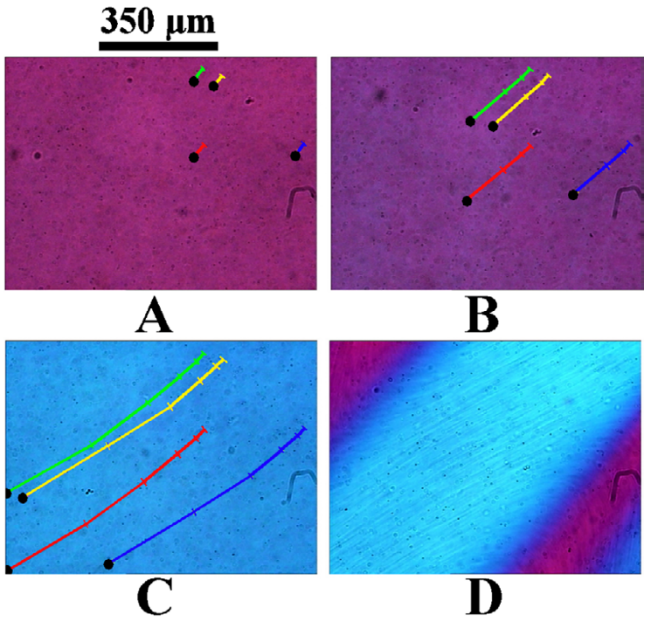

Figure 1
Transport of colloidal polystyrene particles during microtubule self-organisation. Images of the preparation (10 mg ml^{-1} tubulin, 2 mM GTP) as viewed between crossed linear polars and wavelength retardation plate, at different times during self-organisation; A), 20 min; B), 40 min; C), 60 min; D), 5 hours. The numerous small dots are polystyrene beads of 1.1 μm diameter. Several have been highlighted and the coloured lines indicate their trajectories. During the first hour of self-organisation, the microtubules orient along the direction indicated by the bead trajectories. In the completed self-organised structure shown in D), a level of organisation of 20 μm separation can also be seen. At higher magnification, there are other levels of organisation having separations of 5 μm and 1 μm.

added to a final concentration of 5 μM. This concentration is sufficiently low as to not affect either tubulin assembly kinetics or self-organisation. The molecule DAPI possesses the property that it is approximately eight times more fluorescent when associated with microtubules than in the presence of either free tubulin or the buffer solution [19]. The intensity of the light emitted by DAPI when illuminated at its fluorescent excitation frequency is proportional to the microtubule concentration [13].

Microtubules form within 2–3 minutes after warming the solution to 35°C, after which the sample progressively self-organises over about 5 hours as outlined above. Self-organisation was observed by placing the sample between crossed polarisers and a wavelength retardation plate. Macroscopic regions comprised of microtubules oriented at 45° give rise to a blue interference colour whereas regions containing microtubules oriented at 135° are yellow. In each striped band of the self-organised structures shown in Fig. 1 and 3, the microtubules are highly oriented at either 45° or 135°, but adjacent stripes differ by having different orientations.

Although the preparations do not contain molecular motors, approximately 10 minutes after assembling the tubulin into microtubules, all the polystyrene particles in the field of view of the microscope (880 μm by 650 μm) start to move. They all travel in the same direction at a speed of about 1 μm per minute (Fig. 1) and progressively accelerate (Fig. 2) until about 90 minutes later they attain a maximum speed of approximately 5 μm per minute. During this time, the preparation in the observation area becomes uniformly birefringent (Fig. 1), indicating that the microtubules had adopted a uniform orientation. The microtubule orientation was determined as being the same as the direction of particle movement. After this, the striped arrangement described above develops by a process in which microtubules in certain regions progressively modify their orientation. These changes in orientation occur by a mechanism in which the microtubules partially dis-assemble and then re-assemble [12]. During this time, the particle trajectories no longer all follow the same direction over the entire sample, but follow the changes in microtubule orientation as they occur during self-organisation. While this happens, particle movement progressively slows (Fig. 2). Practically no further transport occurs once self-organisation is complete after about 5 hours. Depending on conditions, the average particle speed is between 2 to 5 μm per minute and particles are transported a total distance of between 0.5 to 2 mm. Under highly reactive conditions, the particles sometimes attain velocities of from 10 μm to 20 μm per minute.

During self-organisation, macroscopic differences in microtubule concentration also develop. The concentration pattern that arises coincides with the pattern of birefringence (Fig. 3). Where the microtubule orientation changes from one stripe to the next, the microtubule concentration drops by about 25% and then rises again. During the first part of the self-organising process, all the polystyrene beads move at a similar speed. However, later, as macroscopic variations in sample composition develop, this is no longer the case and a partial accumulation of particles into regions of high microtubule concentration occurs. The particle pattern was observed independently from the microtubule pattern, by recording the fluorescence distribution of fluorescent-labelled beads (Figs. 3 and 4). To be certain that optical effects from the microtubules did not contribute to this image, the preparation was cooled to 7°C on the microscope stage and the microtubules disassembled. There was no

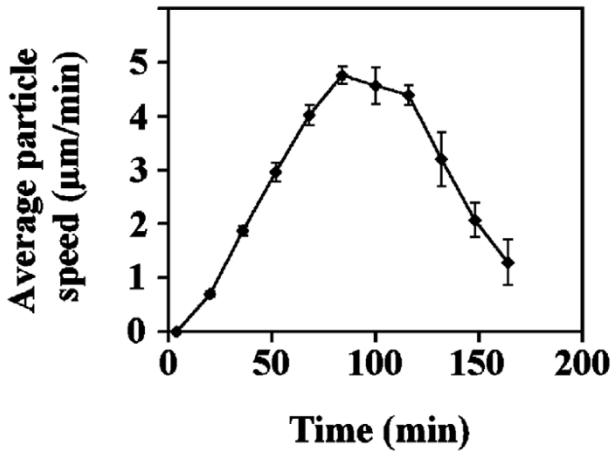

Figure 2
Average speed of 1.1 μm diameter polystyrene particles during microtubule self-organisation. The temperature was 35°C and the tubulin and GTP concentrations were 10 mg ml^{-1} and 2 mM, respectively.

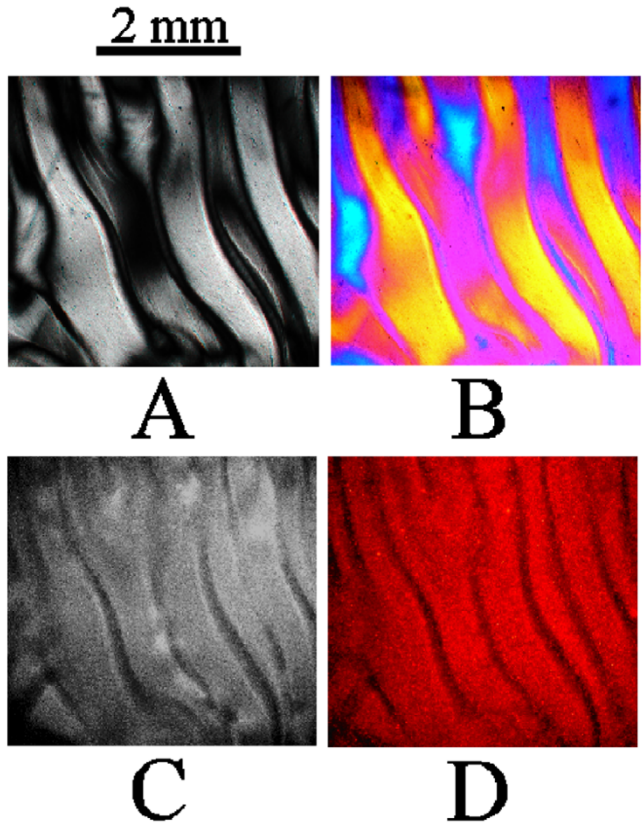

Figure 3
Patterns of microtubule orientation and concentration coincide with that of colloidal particles. The photographs are of the same self-organised preparation but taken using different optical set-ups. A) and B) show the pattern of microtubule orientation. A), is a photograph taken through crossed polars; B), through crossed polars and wavelength retardation plate. C), shows the pattern of microtubule concentration as observed by DAPI fluorescence (excitation 358 nm, emission 451 nm). D) shows the distribution of 1.0 μm diameter fluorescent polystyrene colloidal particles observed by way of their fluorescence (excitation 575 nm, emission 610 nm).

modification in the pattern of fluorescence from the colloidal particles. The distribution of particles, which was initially homogeneous, takes on the same macroscopic pattern as the microtubules. Patterns of microtubule orientation, microtubule concentration, and colloidal particles, observed on the same self-organised preparation are shown in Fig. 3.

Particle movement does not start until well after microtubule assembly is completed. In buffer solutions not containing tubulin, no net displacement of particles occurred, even though in this case the viscosity (10^{-2} poise) is much less than that of the microtubule preparation [20] (10^{4} poise). Self-organisation requires specific microtubule reaction dynamics, and does not occur under all buffer conditions. In particular it does not arise when the buffer described contains either additional magnesium ions (10 mM Mg^{++}) or taxol (0.1 mM). In these cases, although microtubules assemble to the same extent as before, there is no particle transport. This observation suggests that the reaction-diffusion process that give rise to self-organisation also cause particle transport.

Raising either the initial tubulin concentration or the assembly temperature, increase the reaction rates involved in microtubule formation and maintenance. The speed of particle transport increased strongly with increases in both of these parameters (tables 1 and 2). The rate of GTP hydrolysis, as measured by P^{31} NMR [11,12] at different temperatures and tubulin concentrations, concords with the change in particle speed. This data, taken together with the facts that particle transport does not occur in microtubule preparations that do not self-organise, and that particle movement stops when self-organisation is completed, indicate that particle transport is caused by the self-organising process itself.

Experiments were also carried out using in place of polystyrene particles, chromosomes and nuclei isolated [21] from *HeLa* cell cultures at metaphase, and phospholipid vesicles. The latter were comprised of phosphatidyl choline, phosphatidic acid and phosphatidyl serine and

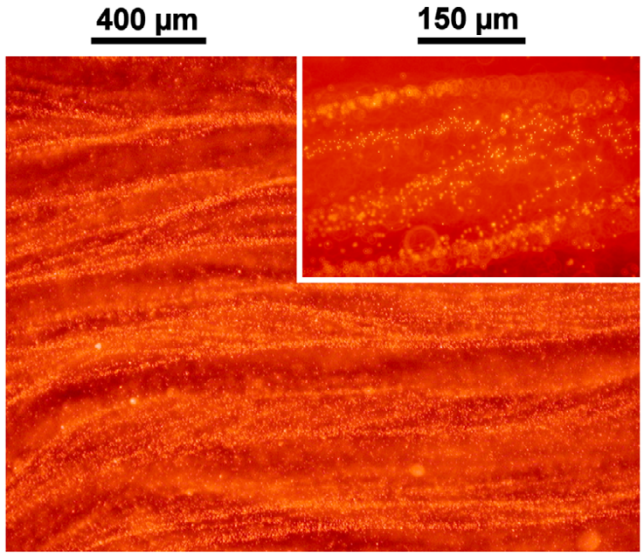

Figure 4
Distribution of 1µm polystyrene particles in a self-organised preparation as viewed by their fluorescence. Microtubules were assembled from a solution of 10 mg ml^{-1} tubulin and 2 mM GTP at 36°C. This pattern coincides with the microtubule pattern. Individual particles are visible at the higher magnification shown in the inset. The particle distribution was homogenous prior to microtubule self-organisation.

Table 1: Average particle speed, 40 minutes after microtubule assembly, of 1.1 µm diameter polystyrene beads as a function of tubulin concentration and the rate of GTP consumption as measured by P^{31} NMR [11,12] at an assembly temperature of 35°C.

Tubulin concentration (mg ml^{-1})	6	10	14	22
Particle speed (µm min^{-1})	1	3	6	7
Rate of GTP consumption (µM min^{-1})	11	16	23	51

Table 2: Average particle speed, 40 minutes after microtubule assembly (tubulin,10 mg ml^{-1}), of 1.1 µm diameter polystyrene beads, as a function of assembly temperature and the rate of GTP consumption.

Temperature°C	30	35	40
Particle speed (µm min^{-1})	0.1	3	7
Rate of GTP consumption (µM min^{-1})	8	16	-

were approximately 1 µm in diameter [22]. These different particles, all moved in the same way as described above and with similar speeds. In the case of chromosomes, an experiment was also carried out in buffer containing 0.1 mM taxol. The microtubule reaction dynamics are now such that self-organisation does not occur and no chromosome transport took place. This observation demonstrates that under appropriate *in vitro* conditions, the presence of taxol can inhibit the movement of chromosomes by microtubules.

Although, the microtubule preparations are highly viscous, one possible manner by which a collective movement of the particles might occur is by a net displacement of the bulk solution such as would arise with thermal convection. To investigate whether this might be the case, the following experiment was carried out. A sample cell was half-filled with a preparation of tubulin and GTP. To it was added, so as not to cause mixing, a solution containing in addition, 95 µM rhodamine chloride and polystyrene beads. Rhodamine chloride is a fluorophore that since it binds to neither tubulin nor microtubules, stains for the bulk solution. The interface between the two solutions was examined under a fluorescent microscope during self-organisation. Initially, all the polystyrene beads and the rhodamine chloride were on one side of the interface. During self-organisation, the polystyrene particles penetrated approximately 200 µm across the interface in tongues about 50 µm wide. This behaviour stopped when self-organisation was complete after 5 hours. The rhodamine chloride fluorescence behaved differently; it progressively crossed the interface both during and after self-organisation, and without forming tongues. This latter behaviour is consistent with molecular diffusion. If flow of the bulk liquid during self-organisation were occurring and displacing the particles, then the rhodamine fluorescence should penetrate across the interface in the same way as the beads. The fact that this is not the case shows that the collective transport of the beads does not arise from flow of the bulk liquid.

Numerical simulations

The question arises as to the molecular basis of how microtubule self-organisation leads to the observed transport behaviour. Although reaction-diffusion processes of the Turing-type lead to self-organisation, they have not been predicted as giving rise to transport properties. The microtubule reaction-diffusion system differs from the normal Turing scheme in that microtubules are chemically anisotropic, growing and shrinking along the direction of their long axes. Experimentally it is extremely difficult to simultaneously monitor and quantify the interdependent behaviour of numerous microtubules in a large population. Hence, to gain insight into the molecular processes underlying the macroscopic transport properties we have carried out numerical simulations of a population of growing and shrinking microtubules using the algorithm previously described. Here, we have

examined the behaviour of the microtubule population at different times during self-organisation. The results suggest that the observed transport phenomena arises from a collective process of microtubule growing and shrinking in which parallel waves of oriented microtubules repeatedly cross the reaction space.

The algorithm used in these simulations has been described in detail elsewhere [16,17]. The parameters incorporated to describe the microtubule reaction dynamics are compatible with experimental values, and the tubulin diffusion constant taken was that measured in the cytoplasm [23]. Briefly, microtubules assemble from tubulin-GTP on a two-dimensional reaction space, measuring about 200 μm by 200 μm, described by a 1 μm grid. Nucleation of microtubules is taken to occur at a rate proportional to the local tubulin-GTP concentration whenever this concentration exceeds a critical value. Individual microtubules subsequently grow and shrink from their ends by the addition and loss of tubulin-GTP and tubulin-GDP respectively. This occurs at rates dependent on the local tubulin-GTP concentration. The concentrations of tubulin-GTP and tubulin-GDP are continually modified at each point on the reaction grid both by tubulin diffusion and by re-conversion of tubulin-GDP to tubulin-GTP. At regular time intervals, the spatial co-ordinates of every microtubule, together with the concentrations of tubulin-GTP and tubulin-GDP, are computed at every position on the grid. The concentration of tubulin-GTP and tubulin-GDP that each microtubule encounters affects the reaction dynamics at both of its ends. These in turn, modify the uptake and liberation of tubulin-GTP and tubulin-GDP at each end. These local concentrations of tubulin-GTP and tubulin-GDP, through diffusion and re-conversion of tubulin-GTP to tubulin-GDP at a given rate, in their turn effect the reaction dynamics at the ends of neighbouring tubes. Nucleation of new microtubules occurs as outlined above wherever the tubulin-GTP concentration locally exceeds a specified value.

As microtubule growth rates increase strongly with increasing tubulin-GTP concentration, we postulate that microtubules will preferentially grow into regions of high tubulin-GTP concentration. To permit this in the algorithm, we made the assumption that microtubules can progressively modify their direction of growth by anything up to a radius of curvature of 3 μm. A consequence of this postulate is that in some parts of the reaction space some of the microtubules will be partially curved. This feature is compatible with experimental observations of the self-organised preparations in which electron microscope images show a significant number of curved microtubules. Moreover, in the regions where the microtubules bend, the maximum radius of curvature is approximately 0.3 μm, significantly smaller than the maximum radius permitted in the numerical algorithm (3 μm).

For suitable values of the reaction dynamics and tubulin diffusion, the reaction space rapidly becomes chemically heterogeneous and self-organisation progressively occurs. The critical parameters required for this, and their values they need to take, are described elsewhere [16,17]. In these numerical simulations, the only hypothesises made are: 1), the reaction scheme outlined above (together with the values describing the microtubule reaction dynamics and tubulin diffusion); and 2), allowing microtubules to progressively change their direction of growth as a function of the neighbouring tubulin-GTP concentration. At any given time in the population of microtubules, depending on the chemical concentrations at each microtubule end, different types of behaviour will occur. Some microtubules will be growing, others will be shrinking; some will be disappearing, other new ones will be forming by nucleation; some will be growing in a straight line whilst others will be growing along a curved trajectory. In this scheme, it is possible to distinguish two possible ways by which self-organisation comes about. One process involves a type of selection in which at different positions suitably oriented microtubules preferentially grow whereas those that are unsuitably orientated do not. The other process depends on the assumption that growing microtubules can change their direction of growth. Both mechanisms operate during, and contribute to, self-organisation. However, in simulations in which microtubules could not modify their direction of growth, macroscopic self-organisation did not come about.

Simulations involving a relatively small number of microtubules illustrate that the growing ends of microtubules grow into the trails of tubulin-GTP produced by their neighbours [16,17]. Groups of neighbouring microtubules progressively grow into the same direction. Once self-organisation is triggered by an external factor that breaks the symmetry of the reaction-diffusion process, then macroscopic rows of aligned microtubules progressively develop. For a large number of microtubules (4.10^4) on a surface 100 μm by 100 μm, a self-organised structure comprised of regular bands of about 5 μm separation develops after 2–3 hours of reaction time. The individual bands are made up of numerous microtubules oriented along the same direction. Between the stripes, the microtubule concentration drops by about 25%. These predictions are in agreement with experiment and the structure compares well with the experimental self-organised arrangement that arises over a similar distance scale.

Fig. 5 shows the results of simulations at different times intervals during self-organisation. These new results show

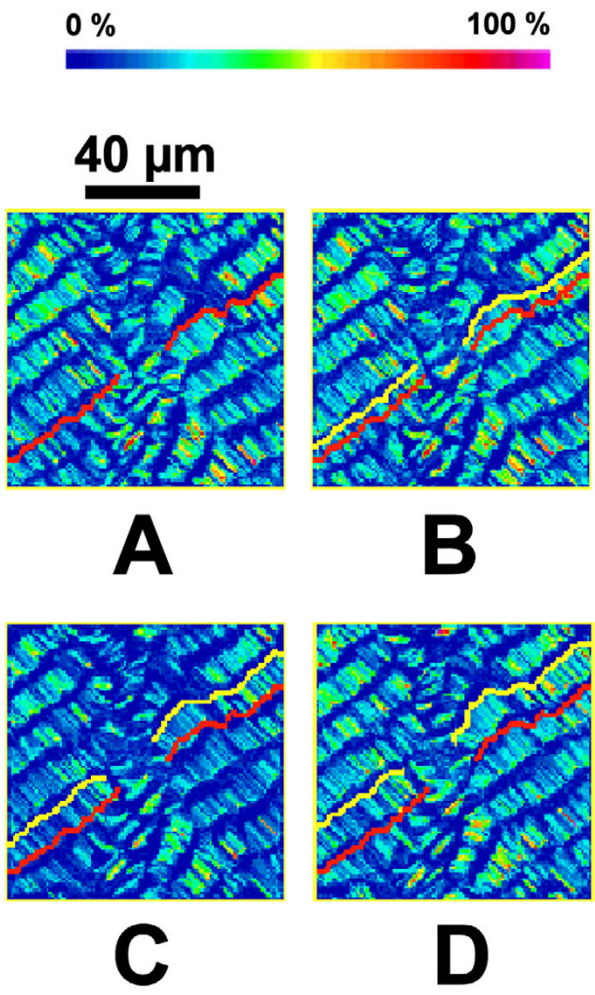

Figure 5
Numerical simulations for a population of microtubules after two hours of reaction time. The reaction space is of two dimensions, measures 100 μm by 100 μm, and contains approximately 4.10^4 microtubules. The simulations shown in A), B), C) and D) correspond to reaction times of 120, 122, 124 and 126 minutes respectively. The colour scale is proportional to microtubule concentration. Self-organisation occurs by the formation of macroscopic arrays of aligned microtubules that advance as collective fronts at a speed of 4 μm min^{-1}. The yellow line highlights the position of one such advancing front compared to its position (indicated by the red line) at the first reaction time shown in A).

that macroscopic arrays of growing and shrinking microtubules move across the reaction space in parallel fronts that change position at a rate of several microns per minute. These waves correspond with variations in microtubule concentration of about 25%. However, they also correspond to large differences in solution viscosity. Hence during self-organisation, repetitive waves both of viscosity and of concentration sweep across the reaction space. These waves will be capable of transporting colloidal particles with them. The fact that their speed and direction is comparable to the speed and direction of particle movement suggests that it is this synchronised collective reactive displacement of numerous microtubules that leads to the experimentally observed particle transport.

The simulations predict that microtubules in an individual array are equidistantly spaced with their growing ends all in the same direction. Individual microtubules, all grow and shrink at the same rate, and so change position simultaneously and advance in a synchronised front. This behaviour comes about in the following way. The growing end of a microtubule gives rise to a region, extending both ahead of itself and down the sides, that is depleted in tubulin-GTP. The growing end of a parallel microtubule slows when it encounters the trail of tubulin depletion caused by a neighbour. In an array of parallel microtubules, any microtubule that grows well out in front of its two neighbours will no longer subject them to the zone of low tubulin-GTP concentration that it causes. The neighbouring microtubules hence grow faster than they would otherwise, and so catch up with the microtubule out in front. As they do this, they again progressively encounter regions low in tubulin-GTP caused by the protruding microtubule and so slow-down again. The growing ends of two neighbouring microtubules synchronised in this way form a pattern of tubulin-GTP depletion that is symmetrical about their mid-point. Hence, the region of highest local tubulin-GTP concentration, which is where another microtubule will preferentially grow or form, is midway between the two. In this way, microtubules in an array all grow at the same rate and are equidistant from one another.

Behind an advancing front, perpendicular to the individual microtubules, a macroscopic tubulin trail arises which is comprised of the sum of the tubulin trails from individual microtubules. Another microtubule array develops just behind this macroscopic tubulin trail and moves along the direction of propagation of the forward tubulin tail. The tubulin rich trail produced by one microtubule array synchronises both the rate and the direction of growth of the microtubules in the following front. In this way, consecutive arrays of large numbers of microtubules arise that advance along the same direction in a synchronised manner.

The simulations are based on the assumptions outlined above; namely, the simplified reaction scheme described (together with appropriate values for the parameters describing the reaction dynamics), and that microtubules

can grow along a slightly curved trajectory. The fact that in experiments a significant numbers of curved microtubules are observed is consistent with this latter hypothesis. One of its consequences is that under appropriate conditions the growing end of a microtubule will grow along the direction and follow the trail of tubulin liberated by the shrinking end of a forward microtubule. Mandelkow *et al* [24] have reported *in vitro* that a new microtubule can grow in exactly the same position as a microtubule which had just disassembled. Likewise, Borisy *et al* [25,26] have observed *in vivo* cases where microtubules follow exactly in the trail of forward microtubules.

There are several reasons to believe that these simulations are a realistic approximation to what actually happens. Firstly, the hypotheses involved are realistic and limited in nature. Secondly, they forecast the main features of the experimental behaviour. They predict not only macroscopic self-organisation in good agreement with experiment but also the fact that self-organisation is triggered by the presence of an external symmetry breaking factor at a critical time early in the process. In experiments, this critical period is both associated with, and coincides with, an instability or 'overshoot' in the microtubule assembly kinetics. The microtubule assembly kinetics predicted by the numerical simulations also show this 'overshoot'. Predicting such a complex experimental behaviour *ab initio* from a simple scheme of microtubule reaction dynamics is neither intuitive nor obvious. The numerical simulations presented in this article illustrate how the same reactive processes that lead to self-organisation can also lead to the observed transport properties. They permit deductions concerning the molecular mechanisms underlying the macroscopic experimental behaviour. At present, it would be extremely difficult to obtain a comparable insight using experimental methods.

Conclusions

Various experiments indicate that the growing ends of microtubules can generate forces capable of displacing intracellular particles [27-29]. Although, this mechanism may displace individual particles over short distances, it provides no in-built manner by which numerous particles are all transported in the same direction over macroscopic distances. For example, in an isotropic solution of microtubules, in which there is no privileged direction of microtubule growth or orientation, there will be no overall direction of particle movement. On the other hand, macroscopic self-organisation by the processes outlined above causes collective transport of numerous particles along a given direction and converts chemical energy into effective mechanical work. In addition, the morphology of the self-organised state, which depends amongst other factors on sample geometry and dimensions, will determine the direction of transport. For example, long elongated forms, such as canals and capillaries, favour a self-organised morphology in which the travelling waves are perpendicular to the long axis. Hence, in elongated cells such as neurones, collective transport of vesicles parallel to the long axis will occur spontaneously.

Reaction-diffusion processes, based upon principles of non-linear chemical dynamics, have often been advanced as a possible underlying mechanism for biological self-organisation [3-6]. However, they are not generally thought of as being capable of giving rise to a collective transport of particles along a given determined direction. The cellular functions of microtubules can be grouped into two categories, organisation and transport. The *in vitro* observations presented here demonstrate that both properties arise simultaneously by the same molecular process. Particle transport, like organisation, arises spontaneously as an 'emergent' property. It occurs by way of the collective behaviour of a population of microtubules in which individual microtubules are coupled to one another by reaction and diffusion.

Microtubule self-organisation, not only transports particles, it also leads to their organisation. As the speed of particle movement depends on microtubule concentration, the development of periodic variations in microtubule concentration results in the progressive accumulation of particles in different regions of the sample. These patterns arise without the need for a specific biochemical interaction between the particles and the microtubules.

Microtubule self-organisation will not only transport particles, it may also transport and organise materials that may be either contained in, or synthesised by, these particles. For example, the process could organise biochemicals contained within vesicles, and which when released might react to form patterns of new products. Microtubules could organise nuclei in such a way that they produce a pattern of gene products.

The combination of microtubule self-organisation, collective transport of particles and particle organisation, is a signature of the microtubule reaction-diffusion process. During cell division, microtubules organise into aligned arrays that transport and separate the chromosomes. In plant cell division, vesicles carrying cell wall precursors move along the direction of orientation of the microtubules, then accumulate, and fuse to form the cell plate [1]. The resemblance between this latter behaviour and the *in vitro* behaviour reported here suggests that microtubule reaction-diffusion processes might play a significant role during cell division. During the blastoderm stage of *drosophila* fruit fly embryogenesis, microtubules transport nuclei to the surface of the embryo [30] whilst simultaneously forming a striped pattern. This pattern

superimposes on that of the gene product engrailed [31,13].

Reaction-diffusion processes of the general type outlined above constitute a novel physical chemical mechanism by which chemical energy is converted into movement. Polymers behaving this way could be used to displace objects in specific directions, or position and organise materials at given locations, and might eventually find application in miniature devices. If so, it would provide an example of how cellular processes might be harnessed to technical applications.

Methods

Tubulin was isolated from cow brains and purified [32] using standard procedures on a phospho-cellulose column. SDS polyacrylamide gel electrophoresis showed no bands other than that from tubulin; in particular there were none from associated proteins of high molecular weight such as molecular motors. Colloidal polystyrene particles of 1.1 μm diameter (Sigma) were added (10^{10} particles ml^{-1}) to preparations of tubulin and GTP (2 mM) contained in a buffer comprised of 100 mM MES (2-N morpholino ethanesulphonic acid), 1 mM EGTA (ethylene glycol-bis-(B-aminoethyl) N, N, N^1, N^1 tetra-acetic acid), and 1 mM $MgCl_2$, in $H^2{}_2O$ at pH 6.75. In this $H^2{}_2O$ based buffer, the microtubules are stable for several days. In some preparations, we used fluorescent-labelled polystyrene beads of 1.0 μm diameter (Sigma). The tubulin concentration was often 10 mg ml^{-1} but some experiments were also carried out at concentrations of 6, 14 and 22 mg ml^{-1}. To some of the samples, DAPI was added to a final concentration of 5 μM. Chromosomes and nuclei were isolated from *HeLa* cell cultures at metaphase using described procedures [21]. Phospholipid vesicles comprised of phosphatidyl choline, phosphatidic acid and phosphatidyl serine were prepared as described [22].

Samples were contained in glass spectrophotometer cells measuring 4 cm by 1 cm by 0.1 cm. Microtubules were formed by removing the sample of tubulin and GTP from an ice bucket and placing it flat down on the hot stage of a polarising microscope (Leitz Pol BK) pre-warmed to, and maintained at the required temperature. The majority of experiments were carried out at 35°C but some were also performed at 30°C and 40°C. Samples were observed between crossed polars (0°, 90°), with a wavelength retardation plate at 45° between them, using a Leitz UB/20 objective. For preparations that are not birefringent, the wavelength plate causes a uniform mauve interference colour. Birefringent preparations containing objects oriented along the direction of the optical axis of the retardation plate produce blue interference colours, whereas those oriented perpendicular to its optical axis show an orange to yellow colour.

Authors' contributions

NG carried out the experiments and the numerical simulations. JD supervised the numerical simulations. JT conceived the study, supervised the experiments, and wrote the article.

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