Biological Self-Organization by Way of Microtubule Reaction−Diffusion Processes†

ARTICLE *in* LANGMUIR · AUGUST 2002

Impact Factor: 4.46 · DOI: 10.1021/la0255875

4 AUTHORS, INCLUDING:

Nicolas Glade
University Joseph Fourier - Grenoble 1
51 PUBLICATIONS **588** CITATIONS
SEE PROFILE

Jacques Demongeot
Le laboratoire AGIM
379 PUBLICATIONS **3,810** CITATIONS
SEE PROFILE

Biological Self-Organization by Way of Microtubule Reaction−Diffusion Processes†

James Tabony,*,‡ Nicolas Glade,‡,§ Jacques Demongeot,§ and Cyril Papaseit‡

Commissariat à l'Energie Atomique, Département Réponse et Dynamique Cellulaire, Laboratoire d'Immunochimie, INSERM U548, D.S.V, C.E.A. Grenoble, 17 rue des Martyrs, 38054 Grenoble Cedex 9, France, and Institut d'Informatique et Mathématiques Appliquées de Grenoble, Laboratoire des Techniques de l'Imagerie, de la Modélisation et de la Cognition, Faculté de Médecine, Domaine de la Merci, 38706 La Tronche Cedex, France

Received January 30, 2002. In Final Form: June 20, 2002

This article addresses the physical chemical processes underlying biological self-organization by which a homogeneous solution of reacting chemicals spontaneously self-organizes. Theoreticians have predicted that self-organization can arise from a coupling of reactive processes with molecular diffusion. In addition, the presence of an external field such as gravity, at a critical moment early in the process may determine the morphology that subsequently develops. The formation, in vitro, of microtubules, a constituent of the cellular skeleton, shows this type of behavior. Preparations spontaneously self-organize by reaction−diffusion, and the morphology that develops depends on the presence of gravity at a critical bifurcation time early in the process. Numerical simulations of a population of microtubules involving only reactive and diffusive terms reproduce this behavior. Microtubules can grow from one end while shrinking from the other. The shrinking end leaves behind itself a chemical trail of high tubulin concentration. Neighboring microtubules preferentially grow into these regions, while avoiding regions of low concentration. The chemical trails produced by individual microtubules thus activate and inhibit the formation of their neighbors, and this progressively leads to self-organization. Gravity acts by way of its directional interaction with the macroscopic density fluctuations present in the solution arising from microtubule disassembly. Evidence is presented that similar processes might occur both during the cell cycle and the early stages of *Drosophila* fruit fly embryogenesis.

1. Introduction

The mechanisms by which biological self-organization occurs from an initially homogeneous solution are not understood. Two possible theoretical approaches might account for biological self-organization. One is based on static interactions and statistical physics between entities that are not involved in chemical or biochemical reactions. The other is based upon nonlinear chemical dynamics and cooperative phenomena involving reacting species. This article is wholly concerned with the latter approach.

Normally, a solution of reacting chemicals or biochemicals does not self-organize. For over 50 years, theoreticians[1−3] have predicted that some particular types of chemical or biochemical reactions that are far-from-equilibrium might exhibit nonlinear properties such as self-organization. In 1940 Rashevsky[1] proposed the counterintuitive idea that in certain types of chemical reactions, diffusion might lead to a partial separation of some of the reactants. In 1952, Turing[2] published a theoretical article predicting that in some reaction schemes, a stationary chemical pattern could spontaneously arise from an initially homogeneous solution. At a molecular level, this behavior arises from a combination of reaction and diffusion, and the patterns that form are made up of periodic variations in the concentration of some of the reactants. Structures of this type are now called reaction−diffusion or Turing structures. They also go under the name of dissipative structures. The latter term was widely used by Prigogine and co-workers[3−5] because a dissipation of chemical energy through the system is required to drive and maintain the system far from equilibrium. This energy dissipation provides the thermodynamic driving force for self-organization.

In addition to self-organization, such systems can also show bifurcation properties.[6,7] At a critical moment early in the process, the system may bifurcate between dynamic pathways leading to self-organized states of different morphology. The presence of a small effect such as an external field, at a bifurcation point of the bistable type, can determine the morphology of the state that subsequently forms. Once the bifurcation has occurred, the system evolves progressively along the selected pathway to the predetermined morphology. It behaves as though it retained a memory of the conditions prevailing at the bifurcation. Kondepudi and Prigogine explicitly calculated that in some reaction−diffusion systems, terrestrial gravity could cause a bifurcation of this type.[8,9]

† This article is part of the special issue of *Langmuir* devoted to the emerging field of self-assembled fibrillar networks.

* To whom correspondence may be addressed. James Tabony, ICH/DRDC, C.E.A. Grenoble, 17 rue des Martyrs, 38054 Grenoble Cedex 9, France. Tel (33) 4 78 38 96 62. Fax (33) 4 78 38 54 83. E-mail jtabony@cea.fr.

‡ Commissariat à l'Energie Atomique, Département Réponse et Dynamique Cellulaire, Laboratoire d'Immunochimie, INSERM U548.

§ Institut d'Informatique et Mathématiques Appliquées de Grenoble, Laboratoire des Techniques de l'Imagerie.

(1) Rashevsky, N. *Bull. Math. Biophys.* **1940**, *2*, 15.
(2) Turing, A. M. *Philos. Trans. R. Soc.* **1952**, *237*, 37.
(3) Glandsdorff, P.; Prigogine I. *Thermodynamic theory of structure, stability and fluctuations*; Wiley: New York, 1971.
(4) Nicolis, G. and Prigogine, I. *Self-organisation in nonequilibrium systems*; Wiley: New York, 1977.
(5) Nicolis, G. and Prigogine, I. *Exploring complexity*; Freeman: New York, 1989.
(6) Thompson, J. M. T. *Instabilities and catastrophes in science and engineering*; Wiley: New York 1982.
(7) Abraham, R., Shaw, C. *Dynamics*; Ariel Press: Santa Cruz, 1983.
(8) Kondepudi, D.; Prigogine, I. *Physica* **1981**, *107A*, 1.
(9) Kondepudi, D.; *Physica* **1982**, *115A*, 552.

10.1021/la0255875 CCC: $22.00
Published on Web 08/13/2002

Rashevsky, Turing, Prigogine et al.,[1−5] and others,[10−12] have proposed that biochemical mechanisms of this type might provide an underlying explanation for biological pattern formation and morphogenesis. These concepts, although a subject of interest and debate, have not been adopted by the majority of chemists and biologists. There are several reasons for this. The first is conceptual; there seems to be a reluctance to adopt the approach that pattern and form can arise from nonlinear chemical dynamics rather than static interactions. However, another more practical reason, is that until 12 years ago, there were no established examples of chemical and biochemical systems known to behave this way. For example, in chemistry, it was not until 1990[13,14] that a variation of the Belousov−Zhabotinsky reaction, originally discovered around 1950,[15] was at last accepted as the first example of a Turing structure. The same situation has prevailed in biology. Until the work reported here, first published in 1990,[16] there were no in vitro examples of a simple biochemical system in a test tube, known to self-organize in the general manner predicted by Rashevsky, Turing, Prigogine, and others.

Microtubules are a major constituent of the cellular skeleton. They play an important role in many fundamental cellular processes and control the organization of the cell interior. Under appropriate conditions, in vitro microtubule preparations spontaneously self-organize by reaction and diffusion.[16−30] They form macroscopic patterns whose overall morphology depends on the gravity direction at a critical moment early in the development of the self-organized state.[19] At this moment, the system bifurcates between two different pathways that subsequently lead to different morphologies. The samples behave as though they retained a memory of their conditions at this critical moment. Experiments under low gravity conditions[24] show that self-organization is triggered by the presence of gravity at the bifurcation time. Other experiments[19,20,21,23] demonstrate that the self-organization contains both reactive and diffusive contributions and arises from collective processes in which assembled microtubules partially disassemble and then reassemble. Computer simulations, which contain only reactive and diffusive terms, of a population of microtubules predict self-organization and bifurcation behavior in agreement with experiment.[27−29]

2. Microtubules

The interior of the cell is organized by the cytoskeleton. The latter is composed of three filamentary components: microtubules, actin, and intermediate filaments. Microtubules are long tubular-shaped objects, with inner and outer diameters of about 16 and 24 nm, respectively.[31,32] They arise from the self-assembly of a protein, tubulin, by way of reactions involving the hydrolysis of a nucleotide, guanosine triphosphate (GTP) to guanosine diphosphate (GDP). Their length is variable; but often they are several micrometers long.

Microtubules have two major roles: they organize the structure of the cytoskeleton, and they permit and control the directional movement of intracellular particles and organelles from one part of the cell to another. They participate in many fundamental cellular functions including, the maintenance of shape, motility, and signal transmission, and play a determining role in the organizational changes that occur during the early stages of embryogenesis. Microtubules are a significant component of brain neuron cells and they make up the mitotic spindles that separate the chromosomes during cell division.

Tubulin has a monomer molecular mass of about 50 kDa, has a diameter of about 4 nm, and occurs as a dimer of the α- and β-monomer forms. When warmed, from about 7 to 35 °C, in the presence of GTP, tubulin assembles into microtubules. During this process, a series of chemical reactions occurs and GTP is hydrolyzed to GDP. Once microtubules are formed, chemical activity continues through processes whereby tubulin is added and lost from the opposing ends of the microtubules by reactions involving GTP hydrolysis. There is hence a continual consumption or dissipation of chemical energy through the system.

One of the particularities of microtubules is that, due to differences in reactivity at opposing ends, they frequently grow from one extremity while shrinking from the other. Since the rates of growth and shrinking are often comparable, individual microtubules change position and appear to move at speeds of several micrometers per minute. The shrinking end of a microtubule leave behind it a chemical trail of high local concentration in tubulin. Likewise, the growing end of a microtubule creates a region depleted in tubulin. Neighboring microtubules will preferentially grow into regions of high tubulin concentration while avoiding the regions of low concentration. The chemical trails produced by individual microtubules activate and inhibit the growth of their neighbors. Thus, neighboring microtubules "talk to each other" by depleting and accentuating the local concentration of active chemicals, and this coupling of reaction with diffusion progressively leads to macroscopic variations in the concentration and orientation of the microtubules.

3. Microtubule Self-Organization

3.1. Stationary Structures. Tubulin solutions at concentrations of the order of 10 mg/mL, made up in a suitable buffer,[16] are assembled into microtubules by warming the solution from 4 to 35 °C in the presence of a large excess of GTP. Microtubules form rapidly within 2−3 min. Progressively, over a period of about 5 h, the

(10) Meinhardt, H. *Models of biological pattern formation*; Academic Press: London, 1982.
(11) Harrison, L. G. *Kinetic theory of living pattern;* Cambridge University Press: Cambridge, 1993.
(12) Cox, E. *Curr. Opin. Gen. Dev.* **1992**, *2*, 647.
(13) Castets, V.; Dulos, E.; Boissonade, J.; de Kepper, P. *Phys. Rev. Lett.* **1990**, *64*, 2953.
(14) Ouyang Q.; Swinney H. *Nature* **1991**, *352*, 610.
(15) (a) Belousov B. P.; 1951, reprinted in *Oscillations and travelling waves in chemical systems*; Field, R., Burger, M., Ed.; Wiley: New York, 1985; pp 605−613. (b) Belousov B. P. *Sb. Ref. Radiats. Med. Medgiz, Moscow* **1958**, 145−147.
(16) Tabony, J.; Job, D. *Nature* **1990**, *346*, 448.
(17) Tabony, J.; Job, D. *Nanobiology* **1992**, *1*, 131.
(18) Tabony, J.; Job, D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6948.
(19) Tabony, J. *Science* **1994**, *264*, 245.
(20) Tabony, J. *Nanobiology* **1996**, *4*, 117.
(21) Tabony, J.; Papaseit, C. *Adv. Struct. Biol.* **1998**, *5*, 43.
(22) Papaseit, C.; Vuillard, L.; Tabony, J. *J. Biophys. Chem.* **1999**, *79*, 33.
(23) Tabony, J.; Vuillard, L.; Papaseit, C. *Adv. Complex Systems* **2000**, *2*, 221.
(24) Papaseit, C.; Pochon, N.; Tabony, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8364.
(25) Tabony J.; Pochon N.; Papaseit, C. *Adv. Space Res.* **2001**, *28*, 529.
(26) Tabony, J.; Glade N.; Papaseit, C.; Demongeot. J. *J. Phys IV* **2001**, *11*, Pr 6−239.
(27) Glade, N.; Tabony, J. *J. Phys. IV* **2001**, *11*, Pr 6−255.
(28) Glade N.; Tabony, J.; Demongeot, J. *C. R. Biol.* **2002**, *325*, 283.
(29) Glade N.; Tabony, J.; Demongeot, J. *Acta Biother.*, in press.
(30) Tabony, J.; Glade, N.; Papaseit, C.; Demongeot, J. *Adv. Space Bio. Med.*, in press.
(31) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. *Molecular biology of the cell;* Garland: New York, 1983.
(32) Dustin, P. *Microtubules*; Springer: Berlin, 1984.

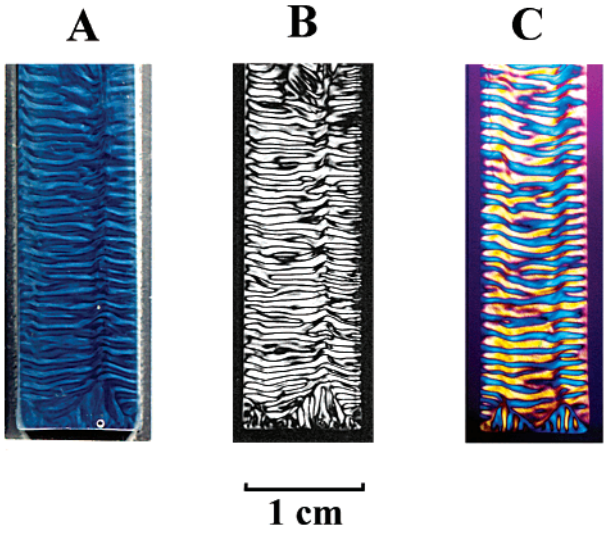

Figure 1. Striped patterns formed by microtubules observed in (A) reflected light, (B) through crossed linear polars at 0° and 90°, and (C) through crossed polars with a wavelength retardation plate at 45°.

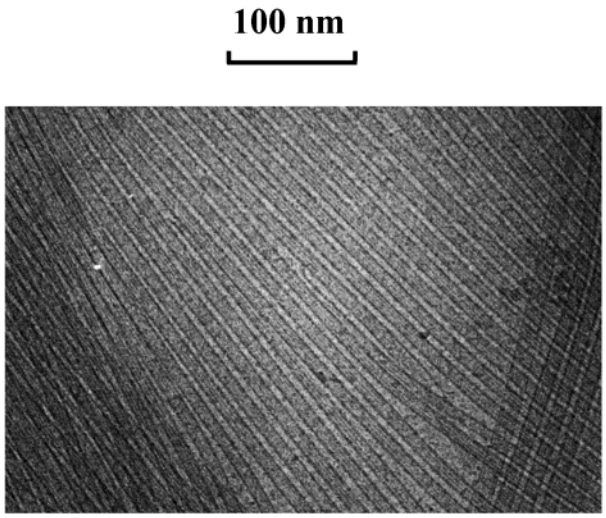

Figure 2. Electron microscope image of part of a self-organized microtubule preparation.

initially homogeneous solution spontaneously self-organizes to form a macroscopic structure. Figure 1 shows the striped arrangement of about 0.5 mm separation that arises when microtubules are assembled in spectrophotometer cells 40 mm by 10 mm by 1 mm. Once formed the structure is stationary, and the solution remains stable for about 3 days.

The preparations are of high optical birefringence, and this shows that the microtubules are strongly oriented over macroscopic distances. Figure 2 shows an electron microscope image in which the arrays of oriented microtubules are clearly visible. The high degree of microtubule orientation can also be observed by way of small-angle neutron scattering measurements.[16,19,21−23] This method, described in another article in this issue, shows that neighboring stripes shown in Figure 1 differ in that the microtubule orientation is either at about 45° or 135°. The striped pattern is hence formed of macroscopic regions in which microtubules are highly aligned at either 45° or 135° but in which the orientation periodically alternates between the two orientations every 0.5 mm up the length of the cell.

This pattern of variations in orientation can also be observed by placing the sample between crossed linear polars with a wavelength retardation plate placed at 45° between the polars (Figure 1c). The retardation plate produces a uniform mauve background. Microtubule orientations, such that their birefringence adds to the birefringence of the wavelength plate, produce a blue wavelength shift, whereas orientations that subtract cause a yellow shift. Sample regions made up of microtubule orientations that are either acute or obtuse differ by producing yellow or blue interference colors, respectively. Due to the alternate periodic variations in the microtubule orientation, the sample when viewed this way appears as a series of alternating yellow and blue stripes.

The structure is considerably more complicated than it appears at first sight. The horizontal 0.5 mm stripes also contain within them another series of stripes of about 100 μm separation. These, in turn, contain another striped structure of about 20 μm separation. At distances below this, there exists another level of organization of about 5 μm periodicity and with care it is possible to observe aligned microtubule bundles separated by about 1 μm. Some of these structures are shown in Figure 3. An additional level of ordering, several millimeters in separation is observed in samples made up in larger sample containers. These large stripes in turn contain the lower levels of organization already mentioned. Hence similar types of patterns spontaneously arise over distances ranging from a few micronmeters up to several centimeters. The range of dimension over which these microtubule structures occur is typical of those found in many types of higher organisms. Cells are about 10 μm in size, eggs are often about a millimeter, and a developing mammalian embryo is several centimeters long.

3.2. Development of the Self-Organized Structure. Figure 4 illustrates the development of the self-organized structure from the initially homogeneous solution. After the solution is warmed to 36 °C, microtubules form within 2−3 min. The first major change occurs after 6 min, with the appearance of a longitudinal bar. This corresponds to a breaking of the symmetry of the solution. As we subsequently discuss, this symmetry breaking is triggered by gravity. It coincides with both an instability in the chemical composition of the sample and a bifurcation between self-organized states of different overall morphology. Just after this symmetry breaking, the sample is divided into two halves on either side of the longitudinal bar. One side of the sample contains microtubules oriented at 45° (blue interference color) whereas the other half contains microtubules oriented at 135° (yellow interference color). Approximately 2 h later, a further break in the symmetry occurs, and a series of stripes perpendicular to the first longitudinal bar begin to develop. These stripes first appear at the sides and then at the top and bottom of the sample. They spread until they progressively encompass the whole cell.

3.3. Self-Organization Results from Chemical Reactions Associated with Microtubule Formation. An important feature to be established is whether self-organization results from the flux of chemical energy through the system or if it arises through static interactions related to the liquid crystalline properties of the microtubule solution. The rate of hydrolysis of GTP to GDP in the preparations can be determined using ^{31}P NMR spectroscopy.[16,19−21,23] Over a period of 24 h there is a progressive reduction in the dissipation of chemical energy through the system. A simple way to test whether the self-organized structure arises from static interactions is the following. Form the striped structure, then destroy it by mixing, and then wait and see whether the structure

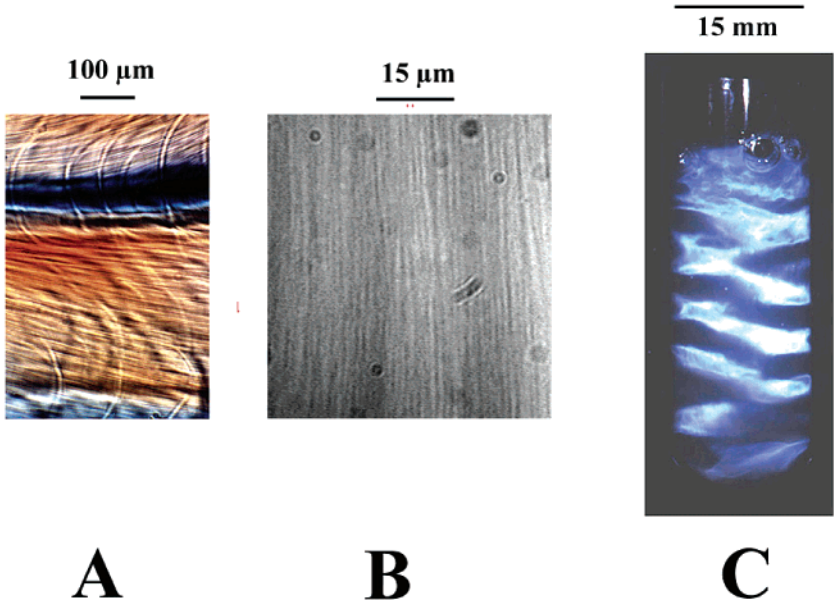

Figure 3. Photographs A and B showing images of a self-organized preparation at higher magnification. Regular separations of approximately, 100 μm, 20 μm and 5 μm and 1 μm arise. Photograph C shows the structure that forms in a 15 mm diameter test tube.

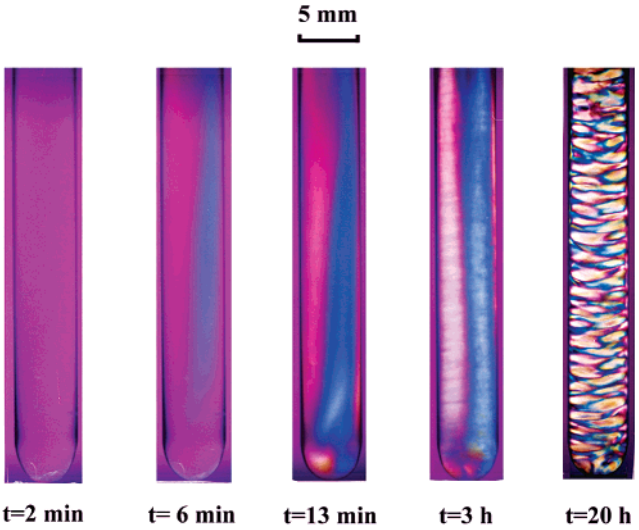

Figure 4. Development of the self-organized microtubule structure in a 5 mm diameter tube. The photographs show the pattern of birefringence at different times (*t*), after instigating microtubule assembly, as observed through cross polars with a wavelength retardation plate.

re-forms.[21,23] After mixing, the solution contains microtubules at the same concentration and temperature as before. The major difference is that at this time, the consumption of chemical energy is significantly less than when the microtubules were initially formed. If the self-organized structure arises from static interactions, such as occur in some liquid crystals, then the structure should completely re-form after mixing. This is not the case.

However, some partial organization does occur. This can be attributed to the chemical activity still present in the preparation after mixing. This activity can be substantially reduced by the addition at the time of mixing of the microtubule drug, taxol. When the energy consumption is further reduced in this manner, no reorganization of the preparation occurs after mixing. After the self-organized structure is formed and then destroyed by mixing, the microtubules can be disassembled by cooling the solution to 4 °C. If afterward, the microtubules are reassembled by rewarming the preparation to 35 °C, then the striped self-organized structure also re-forms.[21,23] These results show that the striped structure arises via chemical processes associated with microtubule formation, and not from static interactions between the microtubules. A further strong argument against static interactions is the dependence of the self-organization on gravity at a critical moment early in the self-organising process.[24,25,30] Static interactions, such as may occur in liquid crystals, are equally present under conditions of weightlessness (0*g*) as at terrestrial gravity (1*g*). The absence of microtubule self-organization under low gravity conditions at the bifurcation time is a clear demonstration that self-organization does not arise from them. The same arguments apply to the possibility that phase separation might be the cause of self-organization, except that in this case the conclusive observation is the bifurcation behavior with respect to gravity. In the case of phase separation, the pattern would not form under low gravity conditions. However, it would form if a brief period of low gravity were followed by 1*g* conditions. As described later, the fact that this does not occur eliminates this possibility.

Another possibility is that the pattern might in some way involve a coupling of the reactive process involved in microtubule formation with convection flow due to thermal gradients. The microtubule preparations are gels of high viscosity (≈5000 P).[33,34] This renders convection difficult. In addition, samples prepared in a hot room at 35 °C, in which we determined there was no convection motion, gave identical patterns to samples prepared under conditions where the bottom of the sample was 5 °C warmer than the top.[18,21,23] It is not necessary to form microtubules by warming a premixed solution from 4 to 35 °C. They can be equally well formed by mixing solutions of tubulin and GTP already prewarmed to 35 °C. The self-organized structure that develops has the same appearance as that formed by mixing tubulin and GTP in the cold and then warming.[19−21,23] Hence thermal convection appears to play no part in the self-organizing process.

(33) Buxbaum, R. E.; Dennerll, T.; Weiss, S.; Heidemann, S. *Science* **1987**, *235*, 1511.
(34) Sata, M.; Schwartz, W.; Selden, S. C.; Pollard, T. D. *J. Cell Biol.* **1988**, *106*, 1205.

3.4. Reactive and Diffusive Contributions to the Self-Organizing Process. One of the important variables in a dissipative system is the rate of energy dissipation. In chemical systems, this is related to the various reaction rates that are strongly dependent upon experimental variables such as concentration and temperature. In a chemically dissipative reaction−diffusion system of the Turing type, the periodicity or wavelength of the structure corresponds to approximately the distance over which groups of molecules diffuse before reacting. The periodicity, L, is related to the reaction rate, R, and diffusion constant, D, by terms involving $L^2 = R/D$.[11,35] In agreement with this, increasing the reaction rate decreases the separation of the stripes by the square root of the increase in the reaction rate.[19]

The rate of diffusion is not varied as readily as the reaction rate. One simple approach is to examine the effect on self-organization of the addition to the initial reaction mixture of small quantities of gelling agents. Increasing quantities of gelling agent increase the viscosity of the preparation and will thus inhibit diffusion. When microtubule assembly is carried out in gels of increasing agarose concentration, the effect is to perturb and eventually inhibit self-organization.[20,21,23] This and other observations concord with a diffusive contribution to the self-organising process.

3.5. Microtubules Partially Disassemble and Reassemble during Self-Organization. During the initial stages of self-organization, the left- and right-hand sides of the cell show either yellow or blue birefringent interference colors corresponding to either obtuse or acute microtubule orientations. The stripes subsequently arise by blue zones forming in the yellow region and yellow zones forming in the blue region. In the zones where there is no color change, the microtubules retain their initial orientation, whereas in the regions where the color changes occur, the microtubule orientation flips from acute to obtuse or vice versa. In neutron small-angle scattering[19−21] measurements of self-organization, in a horizontal band having the approximate dimensions of a stripe, this process is manifested as a change in direction of the microtubular scattering on the detector from an acute to an obtuse arc. Simultaneous with this reordering, the intensity of the microtubule scattering decreases, rises, and then declines again. The microtubule reordering, which is itself the stripe-forming process, is hence concurrent with a chemical wave, involving different concentrations of microtubules and free tubulin, crossing the sample area under investigation. In other words the stationary pattern arises because microtubules disassemble and reassemble with different orientations and concentrations in alternating parts of the sample. This neutron-scattering experiment clearly shows that self-organization results from the reaction dynamics of the microtubules.

3.6. Microtubule Concentration Patterns. The central prediction of reaction−diffusion theories is the formation of macroscopic concentration patterns from an initially homogeneous solution. The images presented above reflect variations in the orientation of the microtubules and not their concentration as such. Small-angle neutron scattering measurements using a small neutron beam were carried out on self-organized microtubule preparations.[22,23] Information as a function of position in the sample was determined by measuring the microtubule scattering in steps along the longitudinal axis perpendicular to the stripes. The changes in microtubule orientation, detected in the neutron measurements as changes in the azimuthal angular distribution, coincide with those seen optically. In addition, the intensity of the scattering taken over all polar angles varied periodically with position. This shows that variations in microtubule concentration occur as a function of position. The concentration is approximately constant in neighboring stripes but drops by about 25% between the stripes, where the orientation changes from acute to obtuse.

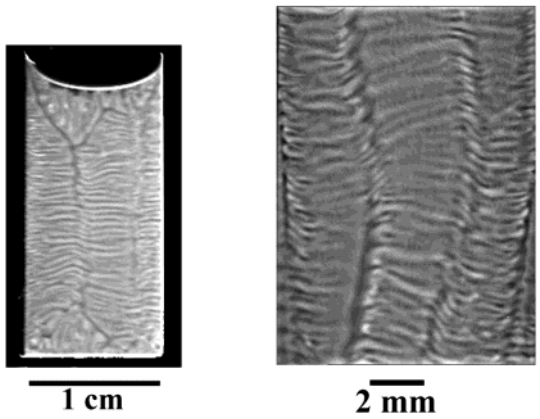

Figure 5. Microtubule concentration patterns as shown by fluorescent imaging.

The fluorophore, 4′,6-diamidino-2-phenylindole (DAPI), is strongly fluorescent when bound to microtubules but weakly fluorescent when associated with free tubulin or in buffer solution.[36] Self-organized microtubule structures were prepared containing small amounts of DAPI. The striped fluorescent pattern shown in Figure 5 arises from periodic variations in microtubule concentration.[22,23] In agreement with the neutron scattering observations, the microtubule concentration drops by about 25% and then rises again whenever the microtubule orientation flips from acute to obtuse. Figure 5 also shows a detail at higher magnification in which the smaller (100 μm) stripes are also visible. These smaller stripes hence also correspond to periodic variations in microtubule concentration.

4. Effect of Gravity on Microtubule Self-Organization

4.1. Effect of the Gravity Direction. The microtubule solutions discussed above were all prepared by assembling tubulin in sample containers that were vertical, and remained vertical, during structure formation. When samples are assembled in the same spectrophotometer cells, but positioned horizontally, face down, a different morphology forms[18−21] (Figure 6b). The patterns of microtubule orientation and concentration vary in a circular manner.

The fact that different types of pattern arise when samples are prepared in optical cells that are either horizontal or vertical shows that gravity contributes to the self-organizing process. To verify this, samples were also prepared with the spectrophotometer cells placed flat down on the turntable of a record player, such that the long axis of the sample cells coincided with the direction of the centrifugal force (0.14g) produced by rotation.[18] As shown in Figure 6C, this resulted in stripes perpendicular to the direction of the applied field. The difference in behavior between the "horizontal" and "vertical" morphologies is hence attributed to the direction of the sample with respect to gravity.

(35) Harrison, L. J. *J. Theor. Biol.* **1987**, *125*, 369.

(36) Bonne, D.; Heusèle, C.; Simon, C.; Pantaloni, D. *J. Biol. Chem.* **1985**, *260*, 2819.

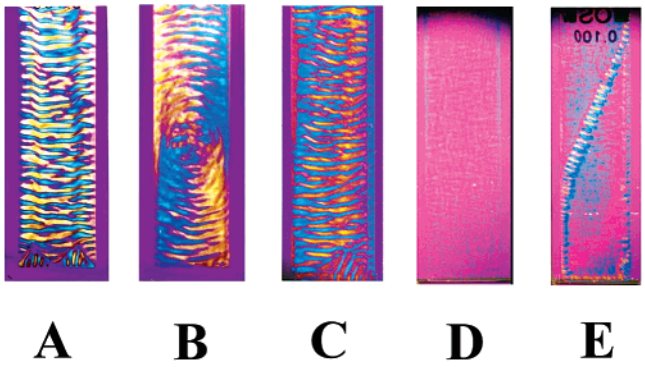

Figure 6. Different stationary morphologies form depending upon gravity vector. In panels A and B, microtubules were assembled in sample cells that were vertical (A) or horizontal (B) during the entire period of structure formation. In panel C they were assembled face down on the turntable of a record player. Panels D and E show microtubule structures as formed during the space flight experiment described in the text. Panel D shows that almost no self-organization occurs for samples subject to weightlessness during the first 13 min of the process. Panel E is a photograph of a sample in which an air bubble in the neck of the cell crossed the sample during payload re-entry.

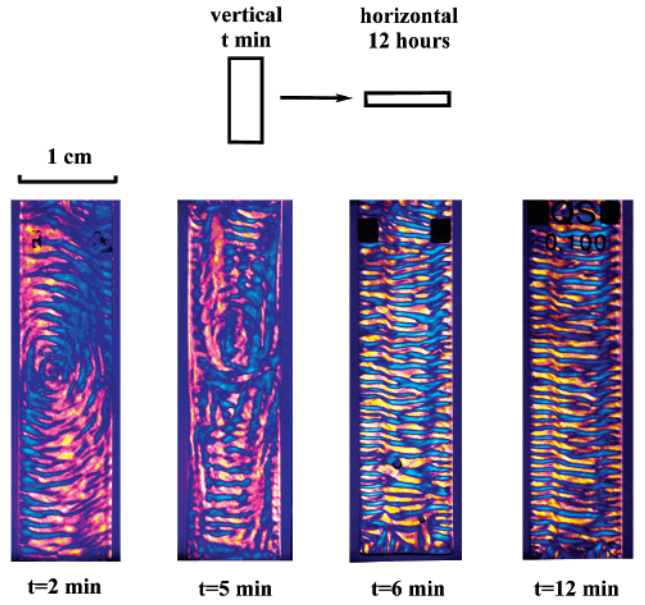

Figure 7. Bifurcation properties of microtubule solutions. The photographs show the final stationary morphologies for samples rotated from upright to horizontal at different times (*t*) during the first 20 min of self-organization. Samples that remained vertical for 6 min or more formed striped structures as though they had remained vertical throughout the entire period of structure formation.

4.2. Morphological Bifurcation. The sample morphology, once formed after 5 h, is independent of whether the samples are horizontal or vertical. So as to determine at what moment in time gravity acts upon the samples, the following experiment was carried out.[19] Twenty samples, made from the same tubulin preparation, were assembled simultaneously in identical optical cells positioned upright. At 1-min intervals, consecutive samples were rotated from vertical to horizontal. The samples were then left undisturbed for about 12 h and examined through crossed polars (Figure 7). As the samples were horizontal at all times except for the first few minutes of the self-organizing process, one might expect that the horizontal morphology would form. This is the case for samples that were turned from vertical to horizontal in the first 4 min after instigating microtubule assembly. On the contrary, samples that remained vertical for 6 min or longer formed striped vertical patterns. Although they were vertical for only a brief initial period, they behaved as though they were vertical for the entire period of the pattern-forming process.

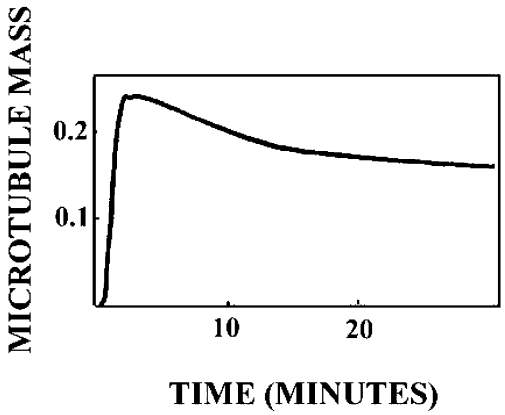

Figure 8. A chemical instability at the bifurcation time is associated with self-organization. The figure shows the kinetics of microtubule assembly as measured by the optical density at 350 nm.

The critical moment at which the morphology becomes determined is 6 ± 1 min after instigating assembly and is prior to the formation of the stripes. At this moment, the system bifurcates between two different pathways that subsequently lead to different morphologies.

The position of the sample with respect to gravity at the bifurcation time determines which morphology forms. The samples behave as though they retained a memory of their conditions at this critical moment. The direction of the weak field at this time results in the formation of a permanently different macroscopic pattern from that which would have arisen for a different field direction.

4.3. Chemical Instability in the Microtubule Solution. For self-organizing processes in far-from-equilibrium systems, bifurcations are associated with the presence of an instability in the initially homogeneous state. For a self-organizing process based on physical mechanisms, the instability involves physical parameters. If self-organization is based on chemical processes, then the instability will involve reactive mechanisms. Hence, establishing whether an instability arises from physical or chemical mechanisms provides evidence as to whether self-organization is due to either physical or chemical factors. In a chemically dissipative system, the instability will involve changes in the concentration of the principal reactants. In the present case, since self-organization results from the reaction dynamics of microtubules from free tubulin, we would expect that a chemical instability involving the relative concentrations of microtubules and free tubulin would occur at the bifurcation time.

The kinetics of microtubule self-assembly can be measured from the optical density of the preparation. Frequently, after an initial increase due to the assembly of the microtubules, the value of the optical density remains approximately constant. In general, microtubule solutions showing this type of behavior do not show any macroscopic self-organization. In the present case, the optical density does not show this type of kinetics. Instead, after an initial rapid increase, it decreases to a value about 20% lower (Figure 8).[19−21,23,24] The maximum in the optical density, and hence in the microtubule concentration, occurs approximately 6 min after warming, at the bifurcation time. There is hence a chemical instability in

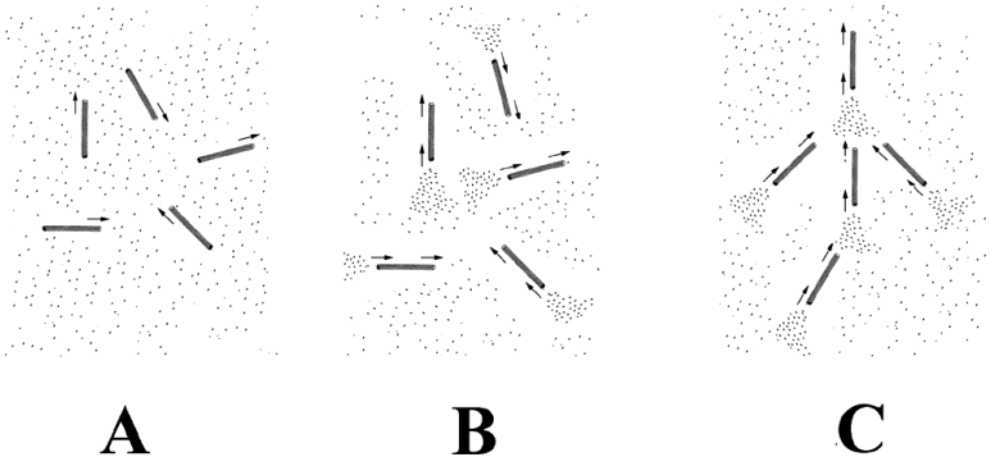

Figure 9. A possible mechanism for the formation of the self-organized structure (see text for details).

the concentration of microtubules at the bifurcation time. In addition, the "overshoot" also corresponds to the first appearance of the longitudinal bar already described. This is the first symmetry-breaking event to occur, and at this point in time the preparation is no longer homogeneous. Just prior to the appearance of this bar, the initially homogeneous solution is unstable.

4.4. Microtubules Assembled under Low Gravity Conditions Do Not Self-Organize. So as to establish whether the self-organizing process is directly dependent on gravity, microtubules were assembled under conditions of weightlessness.[24−27,30] The experiment was carried out during the flight of a sounding rocket of the European Space Agency. The flight provided approximately 13 min of low gravity ($(2 \times 10^{-4})g$) before the payload fell back to earth and was recovered. Samples were contained in an experimental module divided into two compartments. In one compartment, the microtubules were assembled under "low-gravity" conditions, whereas in the other compartment they were assembled on a "1*g*" centrifuge. Hence, samples formed under low gravity conditions could be compared with those formed at 1*g*, under otherwise identical conditions.

During the flight experiment, microtubule assembly occurred. The samples assembled in the centrifuge part of the module self-organized in the manner normally observed in the laboratory. In contrast, those assembled in the low-gravity part of the module show practically no self-organization (Figure 6D).[24−27,30] In addition, they are of weak birefringence, demonstrating that the microtubules do not have any preferred orientation.

Experimental problems frequently occur in space experiments due to air bubbles. In our case, although care was taken to prevent it, in some samples small air bubbles formed in the neck of the sample cell. During re-entry, when the sample is subject to high centrifugal fields, the air bubble is propelled through the sample. In one sample this process was filmed. A line of strong birefringence formed along the trajectory of the air bubble showing that it oriented the microtubules along its trajectory.[26,28,30] Subsequently, striped regions, limited in extent, developed perpendicular to this trajectory (Figure 6E). Hence, orienting some microtubules at an early stage in the process can also trigger self-organization.

5. Molecular Basis for Self-Organization

The reaction scheme for microtubule formation is approximately the following. Tubulin is incorporated into microtubules by way of the complex, tubulin−GTP. This occurs either by addition to a growing end of a microtubule or by nucleation of a new microtubule that subsequently grows. Both processes involve hydrolysis, and the tubulin is incorporated into the microtubule as tubulin−GDP. Likewise, when tubulin is lost from the shrinking end of a microtubule, it is the tubulin−GDP complex that is liberated. When a microtubule grows from one end, while shrinking from the other, it appears to move. The shrinking end of the microtubule leaves behind it a trail of high local concentration of tubulin−GDP. Likewise, as it grows and incorporates tubulin−GTP from the front, it forms a zone that is locally depleted in tubulin−GTP.

The tubulin−GTP complex promotes the growth of microtubules, whereas the tubulin−GDP complex does not. Once liberated, inactive tubulin (tubulin−GDP) is progressively regenerated with a certain time constant, into the active form (tubulin−GTP). At which point, it can again be incorporated into the growing end of a microtubule. During this time, tubulin progressively diffuses out from the trails left by shrinking microtubules. Since rates of chemical reaction increase with increasing concentration, neighboring microtubules will progressively grow along the direction and in the regions where these trails exist. Likewise, microtubule nucleation will be greater in a zone of high tubulin−GTP concentration. These microtubules, in turn, will themselves leave behind, new trails of high tubulin−GDP concentration, and so on. Similarly, the growing ends of microtubules will create regions depleted in tubulin−GTP, and these will behave in a complementary manner. Once regions of increased microtubule concentration and of preferred orientation start to form, then the feedback mechanism outlined above will lead to a progressive reinforcement of this process. In such a way the microtubules "talk to each other", and the coupling of reaction with diffusion progressively leads to a collective behavior resulting in self-organization (Figure 9).

When the microtubules first form from the tubulin solution, the preparation is isotropic (Figure 9A). However, under the conditions outlined above, this isotropic arrangement is unstable (Figure 9B). Once a few microtubules take up a preferred orientation, then neighboring microtubules will also grow into the same direction (Figure 9C). Orientational order thus spreads from neighbor to neighbor, and so on. Hence, at the instability, any small external effect, which leads to the preferential orientation over the whole sample of just a few microtubules, will trigger self-organization.

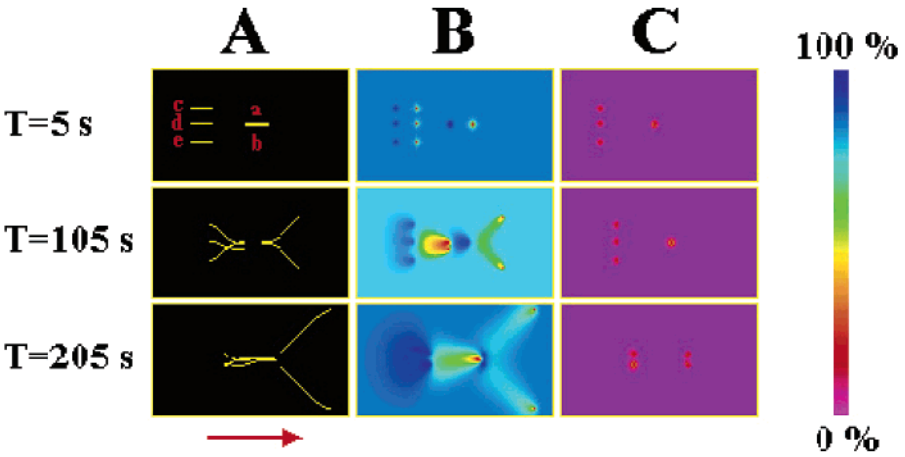

Figure 10. Numerical simulations illustrating the formation of chemical trails: (A) shows the position of the microtubules (a−e), (B) and (C) show the concentration profiles of tubulin−GTP and tubulin−GDP respectively at different reaction times (*T*) during the simulation.

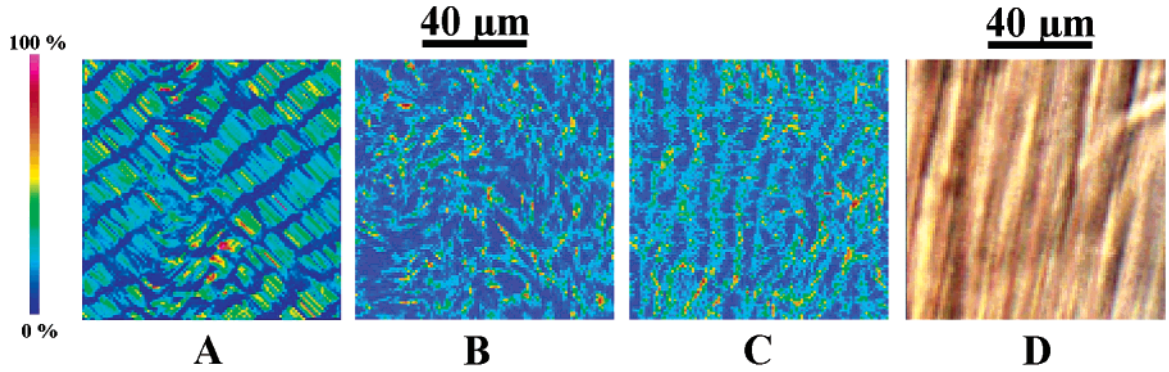

Figure 11. Reaction−diffusion simulations for a population of 5×10^4 microtubules. The diagonal stripes shown in panel A are triggered by a small asymmetry in diffusion. Panel B shows a simulation in which this asymmetry is no longer present. Although concentration inhomogeneities are present, there is no macroscopic self-organization. In panel C the simulation is identical to that in panel B except that diffusion is now twice as fast along the *y*-axis as along the *x*-axis. Self-organized stripes develop in which the microtubules are oriented perpendicular to the direction of the stripes. (D) Experimentally observed self-organized structure over the same distance scale.

6. Numerical Simulations of Microtubule Self-Organization

Numerical simulations[26,28−30] were carried out based upon the premises described above. They used rates of reaction and diffusion within the range of the experimentally determined values found in the literature[29] and reproduced the kinetics of microtubule assembly as observed in the self-organizing process.

One of the things that we wanted to know was whether the chemical trails hypothesized above might be predicted by such numerical simulations. To test this, simulations were carried out with just five microtubules contained on a two-dimensional surface, 100 μm by 60 μm as shown in Figure 10. Figure 10 shows the position of the microtubules and the concentration profiles of tubulin−GTP and tubulin−GDP at different times in the simulation. Due to tubulin−GTP being added at the growing end, and tubulin−GDP being liberated at the shrinking end, all the microtubules progressively change position at a rate of about 5 μm/min. During this process the microtubules form regions, both at the growing end and down the sides, that are depleted in tubulin−GTP. Because of this, the two forward microtubules (a, b) that are close together, grow apart. The shrinking ends of the microtubules liberate tubulin−GDP that is progressively converted to tubulin−GTP. Hence, just behind each microtubule is a small region of high tubulin-GDP concentration. This is followed by a long diffuse trail of tubulin−GTP. The growing ends of the three backward microtubules (c, d, e) grow into the tubulin−GTP trails formed by the forward microtubules (a, b).

We then extended the simulations, to a population of about 5×10^4 microtubules contained on a reaction surface 100 μm by 100 μm. The microtubules that develop attain an average length of about 10 μm, comparable with the experimentally determined value in the self-organized preparation. Likewise in agreement with experiment, the microtubules that first form have no preferred orientation and their distribution is uniform. Subsequently, a self-organized structure comprised of regular bands of about 5 μm separation progressively develops (Figure 11A). Within these bands the microtubules are highly oriented with respect to one another, and the microtubule concentration drops by about 25% in the region between the bands. The structure is hence comparable with the type of structure observed experimentally. Self-organization takes about 10^6 iterations and corresponds to about 2 h of reaction time. In experiments, self-organization takes about 5 h. It was not possible to extend the reaction space to a surface of several square centimeters such as used in the experiments. However, as described above, the self-organized structure contains levels of organization down to distances of about 1 μm. Figure 11 shows a strong similarity between the simulated self-organization and experimental observations over the same distance scale.

One of the aspects of these simulations is that regions of high microtubule concentrations go hand in hand with zones of high tubulin concentration. This means that substantial density differences are present in the preparation. Density differences initially appear when net partial disassembly of microtubules first starts to occur just after the "overshoot". This is hence the first possible moment at which microtubule preparation is capable of interacting with gravity. At this time, the length of the density fluctuation is about 5 μm, and their number density is about 5×10^4 mm^{-2}. The difference between maximum and minimum densities is about 3%. The interaction of such a density fluctuation with a 1*g* field is the equivalent about 30 kT. This is considerably greater than kT, thus demonstrating that the interaction with gravity is sufficient to overcome any thermal averaging in the preparation.

One of the features of these simulations is that the stripes always formed along the diagonal of the square reaction space. This leads us to suspect that an asymmetry favoring this orientation was somehow unwittingly built into the simulation. This turned out to be the case, a very slight asymmetry occurred in the way that diffusion was digitized as a square wave front. When we eliminated it, the simulations show concentration and orientation inhomogeneities of about 5 μm, but which are uncorrelated and disorganized (Figure 11B). When an asymmetry was once again introduced into the simulation by using different rates of diffusion in the x and y directions, then macroscopic self-organization once again developed (Figure 11C).

These numerical simulations demonstrate that a relatively simple scheme, based on experimental rates of reaction and diffusion, predict the overall experimental behavior. They hence permit a link between the microscopic and macroscopic and between the molecular and the phenomenological. The simulations confirm the hypothesis that self-organization arises by the formation of anisotropic chemical trails produced as microtubules grow and shrink from opposing ends. Ordering arises not from static interactions but is due to the formation by reactive processes of concentration gradients in chemicals that activate and inhibit the formation of neighboring microtubules. In this way, microtubules communicate with one another and behave as a macroscopic collective ensemble.

They also predict another major experimental observation: how self-organization is dependent upon weak external fields. Any small effect that creates a slight preference for some microtubules to grow along one direction will trigger macroscopic self-organization. The simulations hence provide a molecular basis for explaining how gravity and shearing can act on the system. Shearing would act by slightly favoring some microtubule orientations, whereas gravity would act by introducing a drift term to molecular transport, equivalent to an asymmetry in molecular diffusion, privileging the growth of some microtubules along the vertical direction.

7. Microtubular Reaction−Diffusion Processes in Vivo

The preceding sections have presented evidence that in- vitro microtubule solutions self-organize by the type of processes initially proposed by Rashevsky,[1] Turing,[2] and Prigogine.[3−5] It has been proposed on many occasions that reaction−diffusion systems might account for some of the global aspects of biological morphogenesis, development, and differentiation. One of the questions that now needs to be answered is whether such processes also occur in vivo. A possible example is the formation of stripes during the early stages of *Drosophila* fly embryogenesis.[37−41] Patterns of gene expression play a major role in setting up the insect body pattern. Another example, is the way that microtubule dissipative systems might account for the dependence of cellular function on weightlessness.

7.1. Space Biology. Experiments in space furnish evidence that various cellular processes, such as growth rates, signaling pathways, and gene expression are substantially modified when placed under conditions of weightlessness.[42−44] In humans, weightlessness depresses the immune system and reduces bone formation. These, and other effects, are thought to arise at a cellular level. However, at the moment there is no coherent explanation for these observations, nor is it known what biomolecules are involved. No cellular component has been identified as having a direct interaction with gravity sufficiently large to affect cellular processes, and biochemical reactions are mostly thought of as being independent of gravity. Nevertheless, many experiments point to an involvement of the cytoskeleton. A possible mechanism by which gravity may intervene in biochemical mechanisms is by way of the bifurcation properties of microtubule reaction−diffusion processes described in this report.

Researchers have observed modifications in the organization of the cytoskeleton[45−48] under low gravity conditions and results on human lymphocyte cells cultured in space show a disorganized microtubule network compared to ground control experiments.[47] Recently,[48] Vassy and co-workers have examined microtubule organization in epithelial cells cultured in space. For many cells, they found that the microtubule network was disorganized (Figure 12). These observations which are consistent with our results, raise the possibility that reaction−diffusion processes form an underlying mechanism for the dependence of cellular function on gravity. However, at the moment the evidence that this is the case is suggestive but not conclusive. Further experiments need to be designed and carried out to demonstrate that in cells the effect of gravity on microtubule organization, arises from the reaction−diffusion processes described in this article. If this turns out to be the case, it would mean that microtubule reaction−diffusion processes occur in living cells.

7.2. Microtubule Stripes during *Drosophila* Fruit Fly Embryogenesis. The early stages of *Drosophila* fruit fly embryogenesis occur by way of consecutive nuclear divisions in a noncompartmentalized egg.[49] It is known that the organization of the cytoplasm by microtubules plays a major role in *Drosophila* morphogenesis.[49] Between nuclear divisions 10 to 14, cells progressively form at the

(37) Kauffman, S. A.; Shymko, R. M.; Trabert, K. *Science* **1978**, *199*, 259.
(38) Harrisson, L. G.; Tan, K. Y. *BioEssays* **1988**, *8*, 118.
(39) Meinhardt, H. *Development* 1988, Suppl. *104*, 95.
(40) Nagorcka, B. N. *J. Theor. Biol.* **1988**, *132*, 277; **1989**, *137* 127.
(41) Hunding, A.; Kauffman, S. A.; Goodwin, B. C. *J. Theor. Biol.* **1990**, *145*, 369.
(42) *Cell and molecular biology in space*, *FASEB J.* 1999, Supplement, *13*.
(43) Hammond, T. G. et al. *Nat. Med.* **1999**, *5*, 359.
(44) Cogoli, A.; Gmünder, F. *Adv. Space Bio. Med.* **1991**, *1*, 183.
(45) Hughes-Fulford, M.; Lewis, M. L. *Exp. Cell Res.* **1996**, *224*, 103.
(46) Cogoli-Greuter, M.; Spano, A.; Sciola, L.; Pippia, P.; Cogoli, A. *J. J. Aerospace Environ. Med.* **1998**, *35*, 27.
(47) Lewis, M. L.; Reynolds, J. L.; Cubano, L. A.; Hatton, J. P.; Lawless, B.; Piepmeier, E. H. *FASEB J.* **1998**, *12*, 1007.
(48) Vassy, J.; Portet, S.; Beil, M.; Millot, G.; Fauvel-Lafeve, F.; Karniguian, A.; Gasset, G.; Irinopoulou, T.; Calvo, F.; Rigaut, J. P.; Schoevaert, D. *FASEB J.* **2001**, *15*, 1104.
(49) Foe V. E.; Alberts B. M. *J. Cell. Sci.* **1983**, *61*, 31.

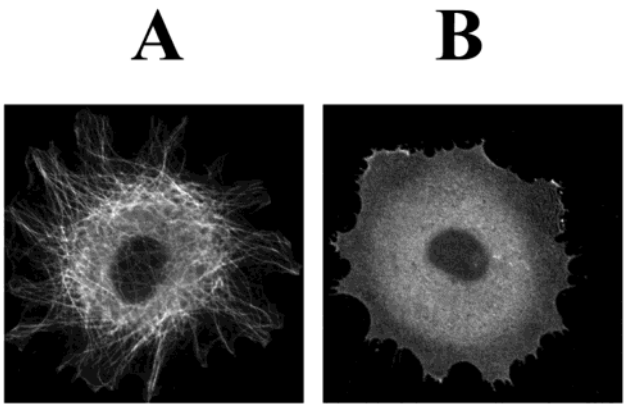

Figure 12. Microtubule organization in human breast cancer (MCF 7) cells: (A) at 1*g*; (B) at 0*g*. As for the in vitro preparations, the microtubules do not organize under low gravity conditions. Published with permission from Vassy et al.[48] Copyright 2001 Federation of American Societies for Experimental Biology.

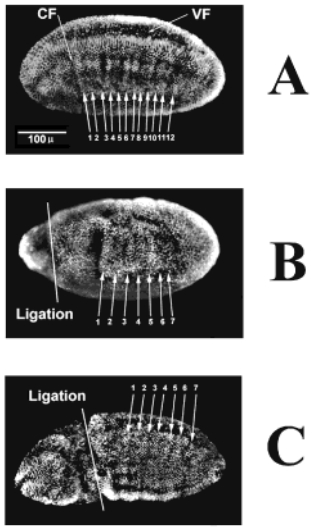

Figure 13. Microtubules patterns observed by immunofluorescence, in (A) whole and (B, C) ligated *Drosophila* eggs. The position of the ventral and cephalic furrows, indicating gastrulation, are shown in (A). Ligation divides the egg into two unconnected fragments, and development continues in one, or both, of the fragments.

surface of the embryo. These cells remain open to the interior of the egg, until the end of the 14th nuclear division. This coincides with the end of gastrulation. Just before this stage, Calliani[50] observed that the distribution of microtubules in the egg forms a striped pattern. The stripes occur in the central part of the egg only; the extremities are not striped. Using the same fluorescence procedure as described by Calliani, we obtained the striped microtubule pattern shown in Figure 13. Although the contrast is low, 12 stripes can be counted, and there may be 2 additional stripes of lower intensity. This striped microtubule morphology appears briefly for around 5 min at gastrulation when the ventral and cephalic furrows appear. It arises, at the same time, and coincides with the pattern formed by the segmentation gene *engrailed*.[51−53] This gene is known to play a role in determining the body segmentation of the larva that develop from the egg.

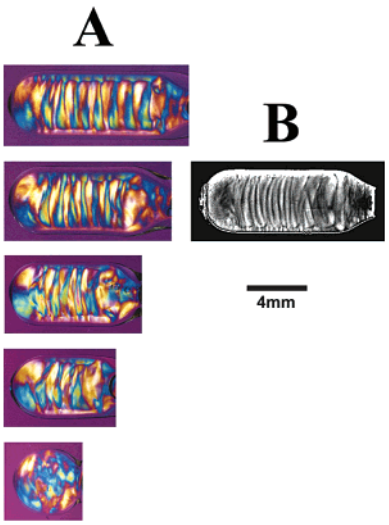

Figure 14. Effect of sample length on microtubule patterns: (A) birefringent patterns formed by microtubules assembled in cylindrical "egg"-shaped sample containers of different length; (B) microtubule concentration variations as detected by DAPI fluorescence. The overall morphology resembles the microtubule pattern in *Drosophila* eggs.

In a reaction diffusion system, in addition to the factors already described, the morphology of the overall pattern may also depend on the dimensions and shape of the sample container. This is due to the boundary conditions in the nonlinear partial differential equations describing the self-organizing process.[3,4,41] When microtubule self-organized structures are prepared in cylindrical containers, whose shape mimic that of a *Drosophila* egg, the morphology shown in Figure 14 arises.[22,23] As for the microtubule pattern in the *Drosophila* embryo, there are two stripe-free regions at each end of the sample, separated by a striped central zone. Below a certain critical sample length, the striped central region does not form. For longer samples, the end zones remain of the same length, and the number of stripes in the central region increases with sample length. For samples of appropriate length, morphologies containing 6 to 7 blue and yellow birefringent stripes arise. When observed by fluorescence, this number doubles to give 12 to 14 microtubule concentration stripes (Figure 14).

Drosophila eggs can be shortened by ligating them shortly after they are layed.[54−57] Within 10 min a membrane forms that separates the egg into two unconnected fragments. Development can occur in either one, or sometimes both, parts of the egg. The dependence of the microtubule pattern on sample length is one of the characteristic features of the in vitro pattern. We hence examined microtubule patterns formed in ligated *Drosophila* eggs as a function of egg fragment length (Figure 13). Eggs were ligated at the 9th or 10th nuclear divisions, 2 h before gastrulation. Although it is not easy to count the exact number of stripes, nevertheless, as for the in vitro microtubule preparation, the microtubule pattern at gastrulation is clearly comprised of two stripe-free extremities separated by a striped central region. Also, as for the in vitro microtubule patterns, the length of the end zones is independent of fragment length and approximately the same as in unligated eggs. In the case of

(50) Callaini, G. *Development* **1989**, *107*, 35.
(51) Nüsslein-Volhard, C. and Weischaus, E. *Nature* **1980**, *287*, 795.
(52) Carroll, S. B and Scott, M. P. *Cell* **1985**, *43*, 70.
(53) Ingham, P. W. *Nature* **1988**, *335*, 25.
(54) Sander, K. *Whilelm Roux's Archiv* **1971**, *167*, 336.
(55) Shubiger, G. *Dev. Biol.* **1976**, *50*, 476.
(56) Shubiger, G.; Moseley R. C.; Wood, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2050.
(57) Boring, L.; Weir, M.; Shubiger, G. *Mech. Dev.* **1993**, *42*, 97.

the ligated egg shown in Figure 13C, both egg fragments have continued to develop. However, the shorter fragment does not show any stripes. This is consistent with the fact that in this case the fragment length is below the critical length necessary for stripe formation.

The behavior of the microtubule pattern in the *Drosophila* eggs resembles that observed for in vitro microtubule self-organization in samples of different length, and this suggests that similar processes might be occurring in both cases. The formation of concentration patterns by reaction−diffusion processes might provide a way by which a predetermined macroscopic pattern spontaneously arises in an initially unstructured egg.

8. Conclusions

The results presented in this article demonstrate that in vitro microtubule preparations self-organize by way of reaction and diffusion. Their behavior is that of a chemically dissipative system in the general framework advanced by Turing,[2] and Prigogine, Glansdorff, and Nicolis.[3−5]

The molecular basis of self-organization is the elongation and contraction of individual microtubules by the addition of tubulin−GTP and loss of tubulin−GDP, respectively. Microtubule contraction leads to the formation of chemical trails of free tubulin−GTP that can be incorporated into other microtubule. Microtubule elongation produces furrows depleted in tubulin. These, in turn, activate and inhibit the formation of neighboring microtubules. Thus neighboring microtubules "talk to each other" by depleting and accentuating the local concentration of active chemicals. The coupling of reaction with diffusion progressively leads to macroscopic variations in the concentration and orientation of the microtubules. This simultaneously gives rise to macroscopic density fluctuations by which self-organization is coupled with and dependent on gravity.

In the present case, self-organization is triggered by the presence of gravity at an early stage of the process. In other words, the system bifurcates due to the effect of gravity. The bifurcation is slightly preceded by a chemical instability in the homogeneous state. The breaking of the symmetry of the homogeneous state at the instability leads to self-organization. The observation that self-organization can also be triggered by the passage of an air bubble through the preparation, strongly suggests that orienting a few microtubules at the moment the isotopic arrangement is unstable, is an essential feature to understanding the role of gravity in the process. Numerical simulations demonstrate how the presence of any small effect in the reactive and transport processes, which lead to a slight directional bias in the distribution of microtubule orientations, will suffice to trigger self-organization.

Cell biologists have invested considerable effort into understanding the processes of microtubule assembly in terms of linear phenomena. The present results suggest that complex biological phenomena can occur as a result of nonlinear reactive processes associated with the formation of microtubules. At this stage it is not established whether these processes are widespread in biology or if they are limited to microtubules. It is quite possible that other reaction−diffusion processes, involving different molecular systems, occur in living organisms. Under appropriate conditions actin will probably show a similar behavior. It may be that the specific type of reaction−diffusion mechanism encountered here, based on reactive growth and shortening of tubes or rods, is a mechanism particularly suited to biological self-organization.

An additional factor is the manner by which such processes might act as a mechanism for biological signal transduction and subsequently lock the system into a developmental pathway. In the present case, gravity triggers the self-organizing process. The gravity direction breaks the symmetry of the initially homogeneous state and leads to the emergence of form and pattern. Such processes may have played a role in the development of life on earth. Other external factors, such as magnetic and electric fields, or shearing, could have the same effect. Processes of this type could form a general class of mechanism by which weak environmental factors are transduced by biological systems. The results summarized in this article demonstrate that gravity substantially modifies microtubule self-organization by way of its participation in a reaction−diffusion process. Gravity can thus intervene in a fundamental cellular process and will indirectly affect other cellular processes that are in turn dependent upon microtubule self-organization.

This approach provides an attractive possible explanation for biological self-organization and development. Self-organization is not the consequence of static interactions but occurs via the chemical dynamics of the system. The attraction of reaction−diffusion dissipative processes as a possible explanation for biological self-organization and development arises not only from their self-organizing properties. It is also due to the fact that their bifurcation behavior could account for some of the global aspects of biological development whereby cells of identical genetic content take different developmental pathways so as to become differentiated from one another. Just after bifurcating, a nonlinear system could be described in biological vocabulary as being "determined but not yet differentiated".

The overall phenomenological behavior of the microtubule preparations shows a qualitative resemblance to some aspects of living organisms in the following ways. First, macroscopic ordering appears spontaneously from an initially homogeneous starting point. Second, the type of morphology formed can depend on small differences in conditions, at a critical moment early in the formation of the self-organized state. Hence, in this system, we notice the emergence of a number of global properties that frequently arise in living systems, namely, self-organization at different distance levels, a dependence on weak effects, and a primitive form of memory.

Rashevsky and Turing first developed their theories as a possible underlying physical−chemical explanation for biological self-organization during embryogenesis. They predicted a way by which macroscopic chemical patterns could spontaneously develop during the early stages of embryogenesis. The results obtained on the in vitro microtubule system demonstrate that reaction−diffusion processes can result in self-organization. The question obviously arises as to whether these processes also occur in vivo; in particular do they occur during embryogenesis and the cell cycle. However, at this stage, although preliminary results suggest that this might well be the case, it is too early to give a definite answer. What we can say is that the approach of out-of-equilibrium chemical dynamics can in principle account for biological self-organization and that an important cellular component, microtubules, behaves this way in a test tube.

These microtubule results demonstrate how a very simple biological system comprised initially of just a protein and a nucleotide, and without DNA, can a show a complex behavior reminiscent of certain aspects of living systems. These complex phenomena which are of considerable biological importance, appear not by way of static interactions but as a consequence of chemically nonlinear dynamics.

Acknowledgment. We thank the Institute Laue-Langevin for access to neutron beam facilities. We thank the European Space Agency for the sounding rocket flight together with the associated experimental flight hardware. We thank the Centre National d'Etudes Spatiales for financial support.

LA0255875