Although weightlessness is known to affect living cells, the manner by which this occurs is unknown. Some reaction-diffusion processes have been theoretically predicted as being gravity-dependent. Microtubules, a major constituent of the cellular cytoskeleton, self-organize in vitro by way of reaction-diffusion processes. To investigate how self-organization depends on gravity, microtubules were assembled under low gravity conditions produced during space flight. Contrary to the samples formed on an in-flight $1 \times g$ centrifuge, the samples prepared in microgravity showed almost no self-organization and were locally disordered.

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This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: DAPI, 4',6-diamidino-2-phenylindole.
When DAPI, at a concentration of 5 μM, is instigated by raising the temperature, then the DAPI fluorescence intensity increases six-fold because of the formation of microtubules (15). Likewise, when the microtubule preparation is cooled to 7°C, the microtubules disassemble, and the DAPI fluorescence decreases to its original low value. Microtubule preparations at the concentration used (10 mg/mL) are highly viscous, 5 × 10⁵ cP (1 P = 0.1 Pa-s) (19), compared with the solution of free tubulin, 1 cP. The solution viscosity thus increases when the microtubules form from tubulin. Likewise, it decreases substantially when the microtubules disassemble in the cold. The same remark applies to the optical density at 350 nm. When microtubule formation occurs, the optical density in the 1-mm-thick sample rises to about 0.2 and drops to a value close to zero when the microtubules are disassembled in the cold.

These experiments clearly establish that, in the self-organized preparations, the tubulin has assembled into microtubules and that these microtubules can be characterized by their optical density at 350 nm, their high viscosity, the intensity of DAPI fluorescence, and the effect that disassembling the microtubules, by cooling the sample, has on these properties.

Before carrying out the space experiment, we were aware that it might be necessary to keep the tubulin preparations for up to 24 h at 7°C before initiating assembly. Hence, on several different occasions, we carried out a series of laboratory tests in which the tubulin was assembled into microtubules after storing the samples at 10°C for 4, 12, 28, 54, and 76 h after preparation, and their behavior was compared with the behavior of the fresh preparation. The sample behavior was monitored by measuring the absorbance curve at 350 nm during both assembly and self-organization, and then for a further 12 h. We also compared the optical birefringence and morphology of the self-organized state with those of the fresh preparation. We found no significant difference between the fresh and stored preparations until the samples had been stored for more than 54 h at 10°C.

The experiment was carried out during the space flight of a MAXUS sounding rocket of the European Space Agency. The flight provides approximately 13 min of low gravity (2 × 10⁻⁴ g) before the payload falls back to earth and is recovered. During reentry, samples are subjected to accelerations from 10 × g to 50 × g for about 3 min. Because, on the ground, the sample morphology is determined by the orientation with respect to gravity 6 min after instigating microtubule assembly, a 13-min flight should suffice to investigate the effect of weightlessness on microtubule self-organization. Electron microscopy also shows that the preparations contain the same microtubule dimensions for up to several days after self-organization (14, 15).
and 5 μm (D). These levels of self-organization did not arise for samples assembled under low gravity conditions.

Fig. 3. On the ground, self-organization also occurs over smaller distance scales. This photograph is taken from a sample prepared in the laboratory and placed between crossed circular polarizers. In addition to the periodicity of about 400 μm (A), it also shows periodicities of about 100 μm (B), 20 μm (C), and 5 μm (D). These levels of self-organization did not arise for samples assembled under low gravity conditions.

the self-organizing process. The possible effect of payload reentry was simulated on the ground by assembling microtubules in cells either vertical or horizontal for 13 min, centrifuging them at 50 × g for approximately 3 min, and then leaving them until the structures formed about 5 h later. The stationary morphologies that arose were the same as for samples left all of the time in the initial sample orientation.

Flight samples were contained in an experimental module (constructed by the Swedish Space Corporation, Solna, Sweden, and Ferrari, Modena, Italy) divided into two almost identical compartments. One, the “low gravity” compartment, contained 8 rectangular cells upright and 11 rectangular cells flat. The other compartment, identically disposed samples were placed on a rotating circular plate producing a 1 × g centrifugal field either along or perpendicular to the long axis of the sample cells. Hence, the samples formed under low gravity conditions could be compared with those formed at 1 × g under otherwise identical conditions. Some samples in both the “centrifuge” and low gravity containers contained 5 μM DAPI.

Results

A ground experiment, the same as the flight experiment, and using the same tubulin preparation, was carried out several days before the launch. Microtubules assembled in the low gravity part of the module, with the sample cells either vertical or horizontal, formed the striped and circular morphologies that normally form in the laboratory (Fig. 1). Preparations lying flat on the centrifuge, and spun with the centrifugal field (1 × g) along the long axis of the cell for the first 13 min only, formed stripes.

For the flight experiment, sample preparations were installed in the payload 14 h before lift-off and were maintained at 7°C. Once low gravity conditions were obtained (2 × 10⁻⁴ × g), the sample temperature was raised to 36°C, thus instigating microtubule formation, and the 1 × g centrifuge was switched on. Just before re-entry, the centrifuge was stopped. Hence, the only difference between the two sets of samples is that one set was subject to weightlessness during the first 13 min. The payload was recovered, and the experimental module was reinstalled in the laboratory at the launch site within 2 h from lift-off. The samples were then left for another 4 h before the module was opened and the samples were examined. The sample temperature was maintained at 36 ± 0.5°C. Fig. 2 shows the results of the flight experiment. The samples formed in the centrifuge part of the module formed stripes when the centrifugal field was parallel to the long axis of the cell, and the circular morphology when the centrifugal field was perpendicular. For the latter samples, the circular pattern is at the bottom of the sample container rather than in the middle. This arises on the ground when microtubules are prepared in samples tilted by 1-2° from the horizontal.

Self organization occurred in both the centrifuge and low gravity compartments of the experimental module during the ground control experiment carried out using the same microtubule preparation as used during the flight experiment. Self organization also occurred during the flight experiment in the “1 × g” compartment. Because previous laboratory studies (10–15) establish that self-organized preparations contain microtubules, then the self-organized preparations formed in the experimental flight module must also be comprised of microtubules. The flight samples were examined in the laboratory 6 h after the launch. All of the samples, including those formed in the low gravity compartment, had almost identical values of optical density at 350 nm, they were all of high viscosity, and the samples containing DAPI all showed a high fluorescence. The
values were, to within experimental error, the same as for samples prepared under normal laboratory conditions and known to contain microtubules. The observations are hence consistent with microtubule formation, for both the $1 \times g$ and low gravity samples. In addition, for both the $1 \times g$ and low gravity samples, after cooling to 7°C, the optical density dropped to a value close to zero; the viscosity fell to a few centipoise; and the DAPI fluorescence intensity dropped by a factor of 6. The low gravity preparations hence had the same characteristic properties, and behaved in the same way under ground conditions that microtubule formation occurs. These observations demonstrate that microtubules formed in both the centrifuge and low gravity compartments during the flight experiment.

The comparison of the $1 \times g$ flight morphologies with those that arise under ground conditions shows that the self-organizing process is unaffected by payload reentry and recovery. In contrast, the samples formed in the low gravity part of the module show practically no self-organization. In samples containing DAPI, the spatial distribution of the fluorescence intensity is proportional to the microtubule concentration distribution in the sample (14, 15). The DAPI fluorescence images for the low gravity samples show an homogeneous microtubule distribution, whereas the $1 \times g$ samples show periodic variations in microtubule concentration identical to the orientational pattern (14, 15). Contrary to the $1 \times g$ samples, the low gravity preparations possess only very weak birefringence, demonstrating that the microtubules do not have any preferred orientation. Observations down to a distance of 10 μm taken under a polarizing microscope showed similarly weak birefringence. Hence, individual microtubules are relatively disordered with respect to one another. This contrasts with the $1 \times g$ preparations that show strong optical birefringence and in which many microtubules are highly oriented along the same direction. The self-organized structures that form on ground contain within the 0.5-mm stripes another series of stripes of about 100-μm separation. These, in their turn, contain another set of stripes of about 20-μm separation. At distances below this, there is another level of organization of about 5-μm periodicity, and with care it is possible to observe aligned microtubule arrays of about 1-μm separation. Some of these periodicities, whose dimensions are comparable with the distance scales frequently encountered in vivo, can be seen in Fig. 3. In the samples assembled under low gravity, there was no evidence for any of these lower levels of organization.

**Discussion**

Static interactions, such as may occur in liquid crystals, are equally present under conditions of weightlessness, as at $1 \times g$. The absence of microtubule self-organization under low gravity conditions is a clear demonstration that self-organization does not arise from such static interactions. Neither does thermal convection play a role in the self-organizing process (11–14). On the contrary, the behavior is in agreement with that of a reaction-diffusion system undergoing a gravity-triggered bifurcation.

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**Fig. 5.** A possible mechanism for the formation of the self-organized structure. Microtubules are chemically anisotropic, growing and shrinking along the direction of their long axis. This leads to the formation of chemical trails, comprised of regions of high and low local tubulin concentration from their shrinking and growing ends respectively. These concentration trails (density fluctuations) are oriented along the direction of the microtubule. Neighboring microtubules will preferentially grow into regions in which the local concentration of tubulin is highest. When molecular transport is isotropic, microtubules grow and shrink equally in all directions, and the tubulin trails retain an isotropic distribution. For self-organization to occur, this symmetry must be broken. Gravity can do this by introducing a molecular transport term that is faster in the up-down direction. This gives rise to a slight directional bias in the formation of chemical trails. Microtubules will subsequently grow and form preferentially along the direction of these tubulin trails. These processes progressively reinforce themselves, resulting in the development of the periodic changes in microtubule orientation and concentration observed. In A, microtubules have just formed from the tubulin solution. They are still in a growing phase and have an isotropic arrangement. In B, microtubule disassembly has started to occur at the bifurcation time. This produces trails of high tubulin concentration from the shrinking ends of the microtubules. These macroscopic concentration fluctuations interact with gravity, denser fluctuations drifting downward and lighter ones upwards, leading to an anisotropy in molecular transport. In C, microtubules are growing and forming preferentially where the concentration of active tubulin is highest. Anisotropic molecular transport at the bifurcation time privileges microtubule growth along specific directions. Once started, this process subsequently mutually reinforces itself with time and leads to self-organization. When gravity is absent, molecular transport remains isotropic, and self-organization is not triggered.
According to Kondepudi and Prigogine (8, 9), in a reaction-diffusion system, the interaction of concentration fluctuations with gravity results in a small directional transport term that destabilizes the equilibrium state at the bifurcation point and thus favors the formation of a macroscopic pattern.

The kinetics of formation of the self-organized microtubule preparations show an over-shoot after about 6 min (Fig. 4). This over-shoot is central to understanding self-organization in these solutions and the way that gravity triggers it. The bifurcation point in any out-of-equilibrium system, and at which point the system is sensitive to weak external fields, must coincide with a condition of instability in the homogenous state. In the microtubule system, the maximum at the over-shoot describes a chemical instability in the relative proportions of free tubulin and microtubules in the preparation. It occurs at around the bifurcation time, when the system depends on gravity. At a molecular level, the over-shoot corresponds to the beginning of a period of overall partial microtubule disassembly. The microtubules assemble and then partially disassemble by about 20% before approaching a stationary value after about 30 min. This occurs by processes similar to those that give rise to periodic oscillations in microtubule assembly (20, 21). Our results show that self-organization does not occur when gravity is absent during the first 13 min.

When the microtubules first form from the tubulin solution, they initially grow with almost no disassembly from shrinking ends. When disassembly does occur, it produces strong macroscopic concentration and density fluctuations between regions of high local tubulin concentration arising from tubulin liberated at the shrinking end of the microtubule, and the region at the growing end of the microtubule that is depleted in tubulin. These fluctuations will have a characteristic size of about the length of a microtubule, 5 μm in the present case. They cannot appear until significant microtubule disassembly occurs. The over-shoot observed in the microtubule assembly kinetics, when overall partial microtubule disassembly starts to occur, is hence the first occasion at which the system can interact with gravity. A hypothetical molecular basis for the way that gravity triggers self-organization is illustrated in Fig. 5.

The results show how a very simple biological system, initially comprised of only tubulin and GTP, is capable of behaving as a gravireceptor. In the present case, gravity triggers the self-organizing process. The gravity direction breaks the symmetry of the initially homogenous 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 presented 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 in their turn depend on microtubule self-organization.

In humans, weightlessness depresses the immune system, reduces bone mass, and leads to various changes associated with aging (22, 23). These, and other effects, are thought to arise at a cellular level, and many experiments point to an involvement of the cytoskeleton (1–3). Workers have observed modifications in cytoskeletal organization (24–26), and recent results on human lymphocyte cells cultured in space show a disorganized microtubule network compared with ground control experiments (26). This observation, which is consistent with our results, raises the possibility that reaction-diffusion processes form an underlying mechanism for the dependence of cellular function on gravity. If this is the case, then it would also mean that microtubule reaction-diffusion processes occur in living cells and may lead to new insights into the physical chemical processes controlling the organization of the cytoskeleton.

We thank the European Space Agency for providing the MAXUS flight together with all of the flight hardware. We thank W. Herfs, R. Demets, G. Frohberg, B. Larsson, and F. Cassese for numerous discussions and for the design and construction of the experimental module. We particularly thank B. Larsson, P. Holm, M. Ludin, K. Loth, F. Cassese, and F. Solieri for their participation in the launch campaign. We thank the Centre National des Etudes Spatiales for financial support and G. Defaye and E. Chambaz for access to laboratory facilities.