MICROTUBULE ASSEMBLY

Switched on with magnets

Magnetic nanoparticles coated with proteins can be used to control the assembly of complex cytoskeletal structures.

Sanjay Kumar

Living cells have a remarkable ability to unleash particular physical and chemical processes at precisely defined times and locations. Critical to this is their ability to locally concentrate and disperse specific effector molecules, which can, for example, selectively bind to a protein and regulate its activity. This natural behaviour has inspired researchers to create a variety of methods to induce the local accumulation of specific molecules and thereby direct their function. Such methods have included the fluidic delivery of small molecules within cells[1,2], the light-induced formation of multimeric proteins[3] and the use of magnetic forces to induce the aggregation of nanoparticles[4–6].

With magnetic forces, one important approach has been to attach effector molecules to superparamagnetic nanoparticles and then reversibly aggregate and disperse the particles by turning an external magnetic field on and off. This allows the local concentration and function of the bound effector molecules to be controlled, and has been used, for example, to trigger signal transduction through the clustering of receptor molecules[5]. This actuation strategy is appealing because it requires no genetic perturbation or photoirradiation and can be carried out at very high throughput, which allows for both single-cell and population-based studies. Moreover, magnetic nanoparticles are easily functionalized, only modestly larger than a single protein and highly biocompatible. In fact, specific nanoparticle formulations are already approved for a variety of clinical applications, which may lower the barrier to eventual translation[7]. Writing in *Nature Nanotechnology*, Zoher Gueroui and colleagues at the École Normale Supérieure in Paris and other institutions in France have now shown that clustering of functionalized magnetic nanoparticles can also be used to locally induce the assembly of complex cytoskeletal structures[8]. The structures can span tens of micrometres in size and influence events that occur far from the site of particle assembly.

The researchers began by covalently attaching a mutant of the protein Ran (Ran GTPase) to magnetic nanoparticles. This protein can stimulate the assembly of microtubules into star-shaped structures called asters in which the microtubules nucleate from a common position and polymerize radially outwards, like spokes in a wheel (Fig. 1). These asters are critical building blocks for microtubule-based spindles, which help segregate chromosomes to daughter cells during cell division. Ran facilitates this process by binding to cargo proteins called importins, causing the importins to release their molecular cargo, which are often proteins that drive aster assembly[8,9]. Therefore, locally concentrating Ran has the effect of locally concentrating these aster-promoting proteins and inducing the formation of asters.

The functionalized nanoparticles were encapsulated in liquid droplets filled with cytoplasmic extract obtained from the oocytes (egg cells) of the frog species *Xenopus laevis*, which is commonly used as a model system for studying cell division. Importantly, this extract contains the full set of macromolecular machinery needed to assemble microtubule asters, making it a very powerful system for testing the regulatory effects of specific factors on aster and spindle assembly. In the absence of an external magnetic field, the nanoparticles remained dispersed. However, when an external magnetic field was applied using a permanent magnet, within seconds the nanoparticles migrated towards the

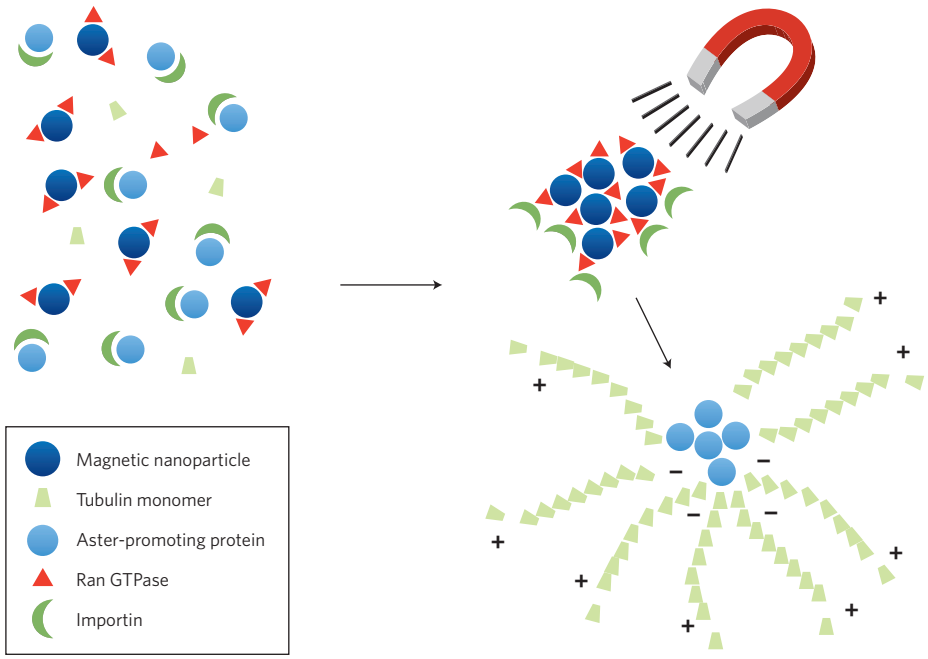

Figure 1 | The assembly of microtubule asters in cytoplasmic extracts using magnetic nanoparticles. The protein Ran GTPase can trigger the assembly of microtubule asters by binding to importin proteins, causing them to release aster-promoting proteins. These proteins directly or indirectly engage with the minus end of tubulin subunits (the proteins that make up microtubules) and nucleate radial microtubule polymerization and aster formation. In the absence of a magnetic field (left), Ran-functionalized nanoparticles are spatially dispersed and the effective Ran concentration is below the threshold needed to assemble an aster. However, on activation of a magnetic field (right), the nanoparticles are magnetized and begin to aggregate, which in turn concentrates the bound Ran molecules. This creates a high local concentration of aster-promoting proteins and leads to the formation of an aster in the vicinity of the nanoparticle aggregate.

droplet boundary and began to aggregate. Within 20 to 30 min, microtubule asters began nucleating near the aggregated nanoparticles. These structures shared the same polarity as microtubules found in the microtubule organizing centres of animal cells (centrosomes), with the polymerization-prone 'plus' ends oriented radially away from the nanoparticles. Notably, aster formation could be induced at bulk Ran concentrations far below what would be needed if the Ran had been freely dispersed in solution.

Gueroui and colleagues also performed a parallel set of studies using nanoparticles conjugated with RCC1, a guanine exchange factor that promotes Ran activation by enhancing the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP), the hydrolysis of which drives Ran function. The magnetically induced aggregation of these nanoparticles also led to the formation of asters. However, this time the asters were frequently located significant distances from the accumulated nanoparticles. To explain this behaviour, the researchers developed a computational model in which RCC1 activates Ran in the vicinity of the nanoparticle and then the activated Ran diffuses some distance before it encounters importin-bound cargo and initiates aster assembly. This 'reaction-diffusion' model was capable of recreating broad features of the data and, most importantly, could explain the differences in induced aster localization between Ran-bound nanoparticles and RCC1-bound nanoparticles. Therefore, by spatially uncoupling the nanoparticle-induced stimulus (RCC1 activation of Ran) from the aster-nucleation process itself, the team were able to extend the spatiotemporal reach of their nanoparticle-induced biochemistry.

Gueroui and colleagues used cytoplasmic extracts bound by liquid droplets as a simple physical model of the cell. This is a reasonable first approximation and could be used to provide other insights into the spatiotemporal course of such signalling events. However, an exciting next step would be to attempt to implement this nanoparticle-based system in living cells. This would of course pose a number of technical and biological challenges. For example, the real cytoplasmic microenvironment is significantly more complex in its structure and organization, and it would presumably be critical to identify some combination of nanoparticle geometry and field strength that minimizes collateral damage as the nanoparticles are dragged through the cytoplasm.

Furthermore, it would be interesting to generalize the method to other cytoskeletal and structural networks within the cell. For example, the assembly of actin-based protein filaments into higher-order structures key to motility and force generation, such as bundles and branched networks, typically requires specific actin-binding proteins. It might be possible to induce directional motility or traction by magnetically clustering nanoparticles coated with these proteins. Indeed, in a separate and equally elegant study reported in *Nature Nanotechnology*, Jacob Piehler, Maxime Dahan and colleagues at the University of Osnabrück, the École Normale Supérieure and the Institut Curie in Paris have shown that magnetically aggregating nanoparticles functionalized with Rho family GTPases can induce the formation of migratory processes in living cells[10].

The potential applications of these nanomagnetic technologies are numerous. For example, the centrosomes found in eukaryotic cells play a critical role in defining cellular polarity and thus the maintenance of a variety of tissue structures. The nanoparticle-nucleated asters have some similarities to these centrosomes and therefore it may be possible to use the approach to drive tissue assembly by guiding the placement of centrosomes. Similarly, this nanomagnetic approach could conceivably be used to control the symmetry of cell division by altering mitotic spindle position. Developing any of these applications will require significant new technologies, as well as sophisticated ways to interface them with cells, but the eventual rewards are considerable. ❐

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FORESIGHT TECHNICAL CONFERENCE 2013

Illuminating Feynman's vision

Chemists, biologists, surface scientists and engineers discuss the many facets of nanotechnology and what 'control' means to them.

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Richard Feynman has a lot to answer for. His vision of atom-by-atom control of manufacturing processes has inspired scientists from across the board to take to the nanoscale world and take on all of the challenges that it involves — although, I am sure, anyone who calls themselves a nanotechnologist or nanoscientist has had days when they have cursed their embracing these tantalizing ideas.

Since its inception in 1986, the Foresight Institute has advocated the theories of the Caltech professor and has been a vociferous presence encouraging and fostering his vision. However, the recent conference entitled 'Illuminating Atomic Precision' held in Palo Alto, California (11–13 January 2013) was significantly different from earlier such conferences in that the organizers — led by the President of the Institute Larry Millstein (Georgetown University) and the co-chair J. Fraser Stoddart (Northwestern University) — opted for decidedly more technical sessions focused on the latest scientific developments rather than emphasize futuristic views of nanotechnology that had characterized previous editions. The conference brought together experts from across many disciplines from academic institutions and industry, and perhaps the most enduring impression that delegates were left with