through the accumulation of freely diffusing proteins, which leads to the nucleation of a distinct body. In that sense, Kaiser and colleagues' tethering experiments may simply recapitulate the immobilization these factors normally experience at their sites of action.

Having said that, the main caveat of this study[1] is uncertainty about its physiological relevance. It is not known whether *de novo* assembly of Cajal bodies occurs naturally, or, if it does, whether it is a frequent occurrence. In fact, Dundr and colleagues previously observed that, when activated, snRNA gene loci can be recruited to pre-existing Cajal bodies[7]. So it is essential now to establish whether *de novo* assembly or recruitment of pre-assembled Cajal bodies is the preferred means of pairing up these structures with their target genes.

Regardless of this, the present work[1] represents a major step forward. Not only does it provide a conceptual framework for probing nuclear-body formation further, but it also gives us a potential means of succeeding in one of the remaining quests of modern cell biology — finding out how cellular structures form *in vivo*. ■

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NANOTECHNOLOGY

Squaring up with polymers

Anthony J. Ryan

Squares may be unfashionable, but for electronic circuitry no other shape will do. A method for making square arrays of polymeric nanoparticles could herald the next generation of miniature silicon chips.

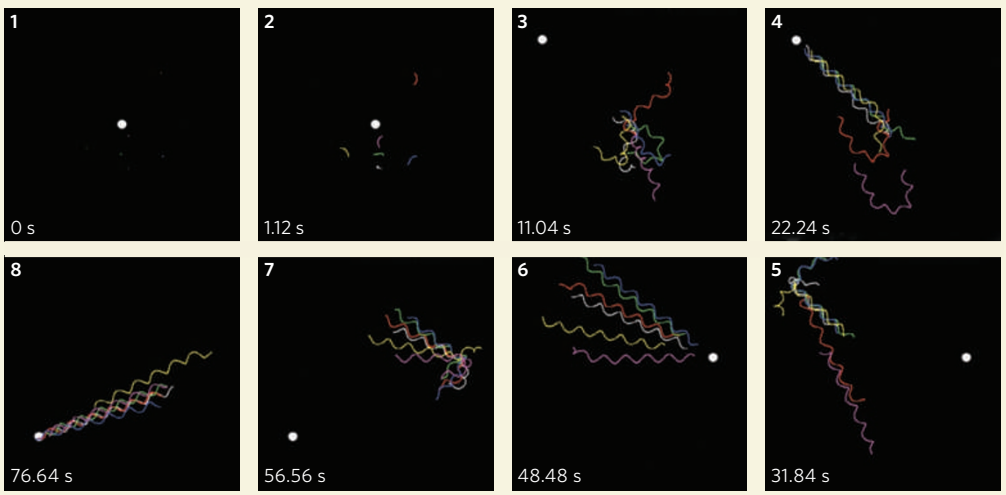
Just ten years ago, who would have thought that you would be able to carry around your entire music collection in your shirt pocket, or your complete genome on a key ring? These amazing feats depend on miniature information-storage devices, which in turn are based on tiny integrated circuits. Although current technology is incapable of scaling down circuits much further, self-assembling nanoparticles could be pressed into service to make much smaller circuitry elements. But there's a problem — nanoparticles self-assemble into hexagonal arrays that are incompatible with the square arrangements used in industry-standard circuits. Reporting in *Science*, Tang *et al.*[1] describe a method in which appropriately designed polymers form nanoparticles that assemble into square arrays.

The tiny structures found in integrated circuits are currently made by photolithography — a technique that uses ultraviolet light to create patterned masks from light-sensitive polymers, which are then used to control the etching of structures into the surfaces of silicon chips. The resolution of this technique is limited to about 100 nanometres (the wavelength of the light used to make the masks). But round nanoparticles can self-assemble into smaller patterns, packing together like oranges in a box. If this process could be used to make smaller features on circuits, it would make a huge difference to the amount of data that can be stored on a chip: a tenfold decrease in feature size could increase information density 1,000-fold.

Self-assembled structures are well known in nature — molecules such as lipids form both nanostructures (vesicles and micelles) and thin films (membranes). The synthetic analogues of lipids are block copolymers. Eleven years ago, it was shown[2] that cheap processes can be devised in which vast arrays of these synthetic polymeric nanoparticles form spontaneously, creating structures ten times smaller than can be made by expensive photolithography. The electronics industry immediately saw the potential benefits of this, but was unable to find a cost-effective way of reworking circuit-design software and fabrication protocols to cope with the hexagonal arrays formed by self-assembly. The Semiconductor Industry

CELL BIOLOGY

Why little swimmers take turns



F. NEDELEC

Gáspár Jékely and colleagues' paper on page 395 literally takes the eye. It provides a neat dissection of how the two eyespots in the larva of a marine worm (*Platynereis dumerilii*) perceive light, and then how the larva responds with differential beating of cilia and a directed, helical swimming action (G. Jékely *et al. Nature* **456,** 395–399; 2008).

These stills come from a dynamic computer simulation, created from first principles, in the authors' Supplementary Information. They show the movement of six virtual larvae that are attracted to a shifting light source; the simulation is initiated in frames 1 and 2, and shows the larval reaction when the light source moves to three different positions (frames 3, 4; then 5, 6; and finally 7, 8). The spiral trajectories traced by each of the organisms closely mirror the behaviour of real *Platynereis* larvae.

This apparently inefficient form of motion, conclude Jékely *et al.*, is optimal for combining eyespot light detection with precise navigation. The authors also point out that at least some of the same principles probably apply to the many members of the zooplankton that swim in spiral fashion.

More about the paper can be found in 'Making the paper' (page xiii), and 'An eye for the eye' (page 304) provides a pictorial gallery of ocular evolution. The computer simulation can be viewed at http://tinyurl.com/59ah73.

Tim Lincoln

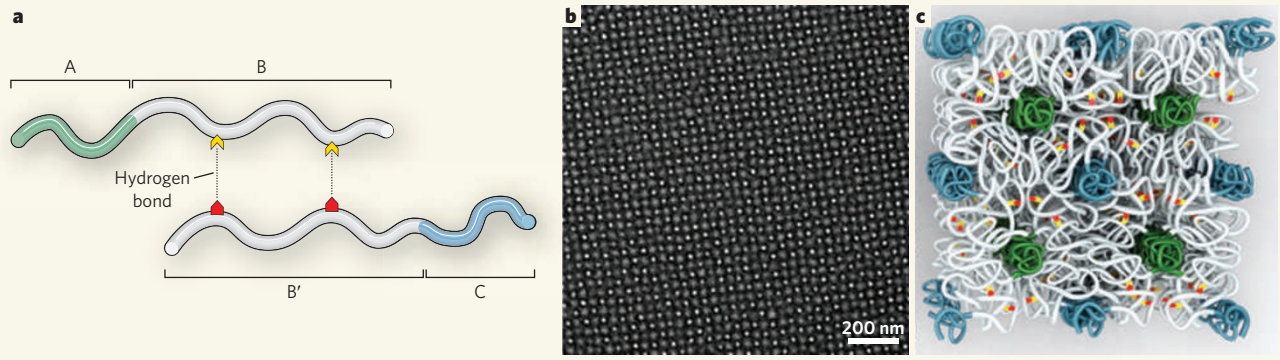


Figure 1 | Square arrays of polymeric nanoparticles. Tang *et al.*[1] have used a blend of diblock copolymers to make a thin film that contains square arrays of cylindrical nanoparticles. **a**, The blend consists of two kinds of diblock copolymer, designated here as A–B and B′–C. The A and C blocks are mutually repulsive and are incompatible with blocks B and B′. Block B contains small numbers of groups (yellow) that form hydrogen bonds to complementary groups (red) in block B′. The two kinds of diblock therefore associate, effectively forming triblock polymers. **b**, This transmission electron microscope image shows that, in a thin film of the blend, blocks A and C form a square lattice of cylindrical nanoparticles (grey and white dots respectively) in a matrix of mixed B and B′ blocks (dark regions). **c**, This cartoon reveals how hydrogen bonding between complementary groups (red and yellow) in the B and B′ blocks (white) hold the diblock molecules together. Cylindrical domains of A blocks (green) and C blocks (blue) form square arrays. (**b**,**c**, Courtesy AAAS.)

Association therefore challenged researchers to develop a method for making square arrays of self-assembled block-copolymer particles. Tang *et al.*[1] have risen to this challenge by using a combination of theoretical predictions and methods for controlling non-covalent interactions between molecules.

Like oil and water, most blends of two different polymers split into separate phases because of nonspecific, dispersive interactions between the two types of molecule. But by linking pairs of different molecules (A and B) with a covalent bond (to form an A–B diblock copolymer), one can prevent phase separation into two layers and gain some control over the nanostructures formed as the molecules aggregate. The nanostructures formed by such copolymers are the result of competition between molecular chain-stretching and interfacial effects around the covalent bond connecting the two polymer domains.

Much is known[3] about the phase behaviour of diblock copolymers. In bulk material, the conditions needed to form three-dimensional cubic structures with long-range order are well established. In thin films deposited on substrates, where the thickness of the film is similar to the spacing between nanoparticles in the arrays, the preferred patterns are: hexagonal arrays of spheres; stripes of cylinders of block A embedded in a matrix of block B (with the stripes laid parallel to the film's surface); or single bilayers (or trilayers) of nanoparticles. The patterns observed depend on the composition of the copolymers and the interactions of the polymer molecules with the substrate and with the air above the film[3].

But more complex molecules, such as A–B–C triblock copolymers — which contain three types of polymer-chain linked in series — can adopt a host of geometries inaccessible to simple A–B diblocks, including chiral and tetragonal arrays formed from cylindrical nanoparticles. Most importantly for forming patterned masks on circuits, A–B–C copolymers form stable, tetragonal arrays of cylinders in thin films. Moreover, the cylinders align perpendicular to the film's surface, unlike A–B diblocks, in which cylinders normally lie parallel to the surface.

There are, however, several problems associated with A–B–C triblocks. Not only are they more difficult to synthesize, but they also suffer from greater packing 'frustration' than do simple A–B diblocks, because the different blocks in each molecule prefer to pack together with blocks of the same type from other molecules. Domains of each block-type thus form, which means that each molecule must pass through two interfaces between different domains.

So although certain triblock compositions can make square arrays in thin films, long-range order is suppressed. Many of these issues can be resolved by making polymer blends of different diblock copolymers (A–B/B–C or A–B/C–D), which provide the advantages of having more than two distinct polymer blocks while avoiding the problems associated with connecting all the blocks in one molecule. But it has proved impossible to achieve long-range order in such blends because of their overwhelming propensity to generate separate phases, just as homopolymers do.

Tang *et al.*[1] overcome this problem by using hydrogen bonding between a pair of A–B/B′–C diblocks. In their blend, blocks A and C are chemically different, and both are mutually incompatible with the B and B′ blocks. The authors' neat trick is to introduce small amounts of complementary hydrogen-bonding groups into the B and B′ blocks (Fig. 1a), so that the blocks form hydrogen bonds to each other. The resulting complex of diblocks behaves like a triblock, and phase separation of the diblocks is suppressed because the hydrogen bonding overcomes the nonspecific dispersive interactions.

The authors made their diblock copolymers using state-of-the-art methods to accurately control both the molecular weight of each block and the number of incorporated hydrogen-bonding groups. They then prepared the A–B/B′–C blend simply by mixing solutions of the two diblocks, and created thin films of the blend (about 50 nanometres thick) on silicon wafers. Using atomic force microscopy (AFM) to observe the nanoscale structure of the films, the authors showed that cylindrical nanoparticles in the film formed perpendicular, square arrays with long-range order (over an area of about 5 × 5 micrometres; Fig. 1b).

They obtained the best results when nearly equal numbers of complementary hydrogen-bonding units were incorporated into the B and B′ blocks. Blends that lacked complementary hydrogen-bonding groups formed only small nanostructured regions, which had hexagonal local order. The lack of nanostructure formation in such blends is probably caused by phase separation of the diblocks.

Tang and colleagues' numerical simulations[1] of the packing in their blend reveal that square-packed cylinders have a lower free energy than hexagonally packed cylinders, unlike pure A–B diblocks. This is because a square lattice allows a more uniform distribution of C blocks around each A block (and vice versa), which minimizes unfavourable stretching of the B blocks even though the cylinders are not packed as closely as they could be. Although the A and C units are not connected on the same chain, dynamic hydrogen bonding ensures that, on average, an A–B diblock is always connected to at least one B′–C diblock. This favours B–B′ mixing at uniform chain-stretching, so that the blend mimics the behaviour of A–B–C triblocks.

The authors used their block-copolymer films as lithographic masks to transfer a template image into a silicon substrate. Using

scanning electron microscopy, they observed that cylindrical pores (with diameters of about 22 nanometres) were formed in the substrate, spaced 50 nanometres apart. This was consistent with the patterns seen by AFM in the freshly formed films, and demonstrated a high degree of fidelity in the pattern-transfer process.

Could Tang and colleagues' approach be used in the mass production of integrated circuits? IBM already use block copolymers as masks for photolithography to make hexagonal arrays of cylindrical pores in insulators, so it seems that industry could easily adopt the authors' method. But one inherent problem is that of residual structural defects, especially when using large films — the larger the area covered by a mask, the more likely it is that a defect will occur on the underlying wafer. One solution could be to make mosaics of films from submillimetre-sized patches of polymers, so that the discovery of a defect doesn't require the whole wafer to be rejected; instead, any device using the wafer can be programmed to avoid using the defective 'tile'.

A combination of Tang and colleagues' technique with the latest technologies for making patterned diblock copolymers on patches and in channels could be used to make arrays of magnets that store trillions of bits of information per square centimetre. But this would be only the start: further developments will undoubtedly lead to greater miniaturization of silicon chips, and to the creation of electronic devices powerful beyond our imagination. ■

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PHYSIOLOGY

Courier service for ammonia

Mark A. Knepper

Physiological studies in mice demonstrate a surprising role for a kidney protein related to the rhesus factor of red blood cells. Similar research would aid further annotation of mammalian genomes.

The completion of the Human Genome Project spawned a new era of biological research, with many declaring that we have now entered the 'post-genomic' era. This declaration is premature, not least because comprehensive functional annotations are lacking for most of the 20,000-odd protein-coding genes in the human genome[1]. Without this knowledge, we cannot gain a thorough understanding of human disease. So a crucial task ahead is to discover the *in vivo* function of each gene product. By investigating the function of the protein encoded by a gene called *Rhcg*, using classical physiological techniques in mice, Biver and colleagues[2] (page 339 of this issue) provide a model for how this goal can be effectively pursued.

We are constantly facing an acid threat. When oxidized in the body, excess dietary proteins produce sulphuric and phosphoric acids, posing the danger of a lethal drop in blood pH. But kidneys prevent our demise by removing these acids through the excretion of hydrogen ions (H^+). Most of this H^+ is excreted in the urine as ammonium (NH_4^+), which is made by cells in the proximal tubule of the kidney from the amino acid, glutamine.

From the proximal tubule, a series of NH_4^+-transport processes, ending with a transport step across the cells of the collecting duct, ensure the transfer of NH_4^+ to the urine[3] (Fig. 1). In the final step, NH_4^+ is believed to enter the collecting-duct cells by direct transport[4], but leaves these cells by the parallel transport of its constituents — H^+ and ammonia (NH_3) — into the urine[5]. An ion pump called V-ATPase mediates H^+ transport. As for the ammonia, because of its small size (17 daltons), it was believed to diffuse freely across the membranes of collecting-duct epithelial cells into the urine, independently of a transporter protein[5]. But is this really the case? Biver *et al.*[2] suspected otherwise, and so tested the hypothesis that the protein product of *Rhcg* is responsible for ammonia transport out of collecting-duct cells.

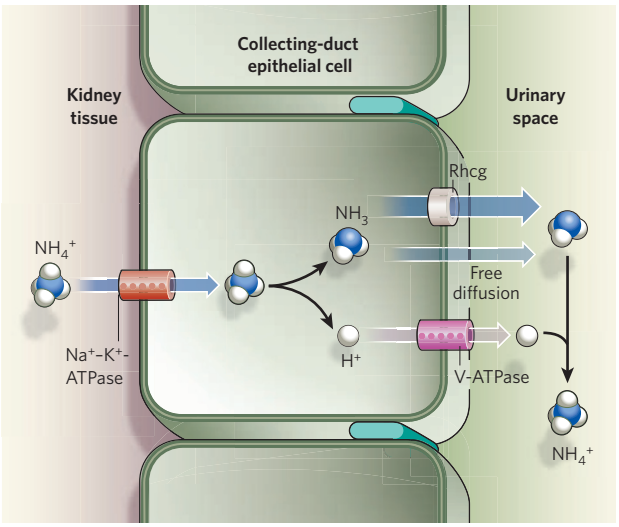

Figure 1 | Ammonium excretion. Acids generated from the metabolism of excess dietary protein are excreted largely as urinary ammonium (NH_4^+) produced by cells in the proximal tubule of the kidney. NH_4^+ is ultimately transported into the urine from collecting-duct cells through parallel movement of hydrogen ions (H^+) and ammonia (NH_3). Biver *et al.*[2] show that not all ammonia moves by free diffusion — as thought previously — and that most of it crosses through the Rhcg protein, which functions as an ammonia channel. The V-ATPase pump mediates H^+ transport into the urine, where it recombines with NH_3 to form NH_4^+. As for the initial NH_4^+ entry into these cells, an ion pump called Na^+–K^+-ATPase, which can carry NH_4^+ in lieu of potassium, has been proposed to be involved[4].

The protein in question, Rhcg, is related to the rhesus (Rh) antigen protein of red blood cells that is used to determine immunological compatibility before blood transfusions. It had previously been shown[6] that the amino-acid sequences of mammalian Rh-family proteins are similar to those of known ammonia-transport proteins of bacteria, fungi, plants and invertebrates. Indeed, when investigators expressed mammalian Rhcg in cells that do not normally express it, they detected transport of ammonia[7]. But this observation is not a definitive proof for the physiological role of Rhcg at its natural levels in mammalian tissues. What's more, when *Rhbg* — the gene for another Rh-related protein of the collecting duct — was deleted in mice, no measurable defects were detected in ammonia transport in the kidneys[8].

Undaunted by the negative result with *Rhbg*, Biver *et al.*[2] deleted the *Rhcg* gene in mice. The authors demonstrate that, contrary to the free-diffusion hypothesis[5], Rhcg absence results in a two-thirds reduction in the rate of ammonia movement across the collecting-duct epithelial cells. This indicates that, at most, only one-third of ammonia crosses the lipid membrane by diffusion. So Rhcg seems to function as a membrane channel, allowing the passage of ammonia across the lipid membrane between collecting-duct cells and the urine (Fig. 1). The absence of this channel in the *Rhcg*-knockout mice results in decreased excretion of ammonia in the urine and a marked drop in blood pH, clearly establishing a role for Rhcg in the regulation of pH in body fluids.

Biver and colleagues also report a reduction in reproductive capability of male *Rhcg*-knockout mice, associated with altered epididymal function. Like the kidney's collecting duct, the