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Research Focus

Star Trek replicators and diatom nanotechnology

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Diatoms are single celled algae, the 10^5–10^6 species of which create a wide variety of three-dimensional amorphous silica shells. If we could get them to produce useful structures, perhaps by compustat selection experiments (i.e. forced evolution of development or evodevo), their exponential growth in suspension cultures could compete with the lithography techniques of present day nanotechnology, which have limited 3D capabilities. Alternatively, their fine detail could be used for templates for MEMS (micro electro mechanical systems), or their silica deposition systems isolated for guiding silica deposition. A recent paper has demonstrated that silica can be replaced atom for atom without change of shape – a step towards the Star Trek replicator.

Given that diatoms are responsible for ~25% of the world's net primary production [1] they have been the most underfunded organism per unit mass. This might be about to change with a flurry of activity in a new field dubbed 'diatom nanotechnology', which will have its debut in a workshop at the next North American Diatom Society Meeting, (see http://serc.fiu.edu/periphyton/NADS/Homepage.html and the forthcoming special issue of *J. Nanosci. Nanotech.*).

Paid and unpaid (so-called 'amateur') diatomists have spent lifetimes doing research on these single-celled creatures, with perhaps one universal motivation: they are beautiful to behold (Figs 1a–c and Fig. 2). Some investigators got carried away, producing art only visible through a microscope, made of arrays of diatom valves (see http://thalassa.gso.uri.edu/flora/arranged.htm).

Diatom motility

At first, diatoms were thought to be animals (A.P. Ussing *et al.*, in preparation) because what are now called raphid diatoms move at speeds of up to 25 μm s^{-1} when attached to surfaces. They do this with no moving parts, although a 'raphe fluid' is exuded. A slow moving debate has occurred over three decades as to whether the motive force for diatom gliding motility is in the hydration and capillarity of the raphe fluid [2] controlled by actin [3], or whether actin provides the motive force [4]. A model for myxobacterium motility identical to the capillarity model for diatoms has been proposed [5], as well as a similar one for cyanobacterial gliding motility [6] (common ancestor or convergent evolution?). Furthermore, some diatoms use 'nozzles' (pore openings) analogous to those on myxobacteria, to secrete an adhesive material [7], which involves transient motility [8]. Definitive experiments are needed because control of the diatom motor, which can reverse rapidly 'on a dime', somehow detecting a collision or a change in light [9], is a fascinating problem of potentially universal significance. Control of motility of diatoms in rigid [10] shells would appear to be simpler than crawling of flexible tissue culture cells of ever-changing shape, and

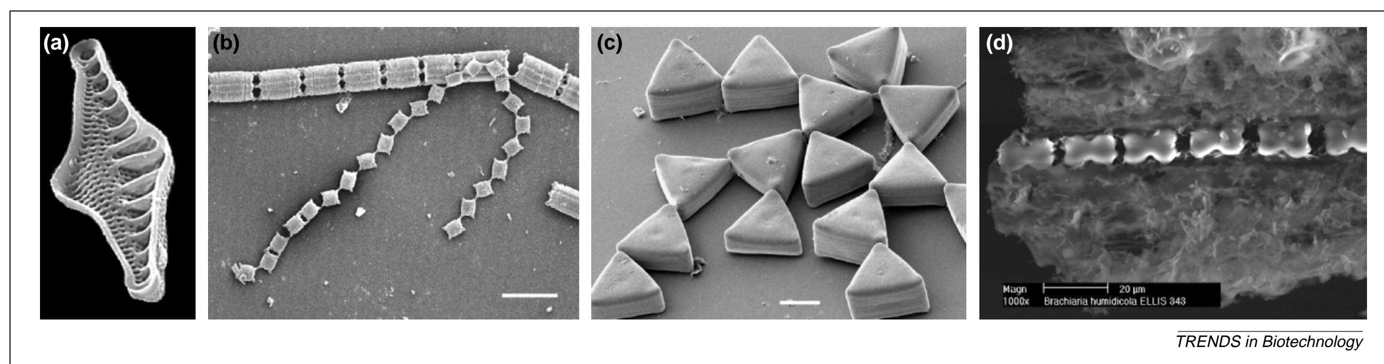

Fig. 1. (a) Scanning electron micrograph (SEM) of the inside of one valve (shell) of the pennate diatom *Nitzschia* sp. about 20 μm long (see http://www.bgsu.edu/departments/biology/facilities/algae/SEM/nitz1.gif, with permission granted by Rex L. Lowe, Center for Algal Microscopy and Image Digitization, Bowling Green State University, OH, USA). The cross ribs form perpendicular to the long midrib, and if caught early by silica starvation, could remain as a comb. Most other pennates are approximately left–right symmetric. Note the pores, which form last. (b) Chain diatoms, *Biddulphia biddulphiana* (top) and *Odontella aurita*; scale bar 200 μm. (c) Triangular diatoms, *Trigonium arcticum*; scale bar 100 μm. (b,c) Taken from Point Loma, CA, USA and provided courtesy of Mary Ann Tiffany, Center for Inland Waters, San Diego State University, CA, USA). (d) Scanned electron micrograph of dry-ashed leaf of 'coronivia grass' *Brachiaria humidicola* showing its silica bodies; scale bar 20 μm (permission of Christian Mulder, Paleobotany and Palynology, Utrecht University, The Netherlands).

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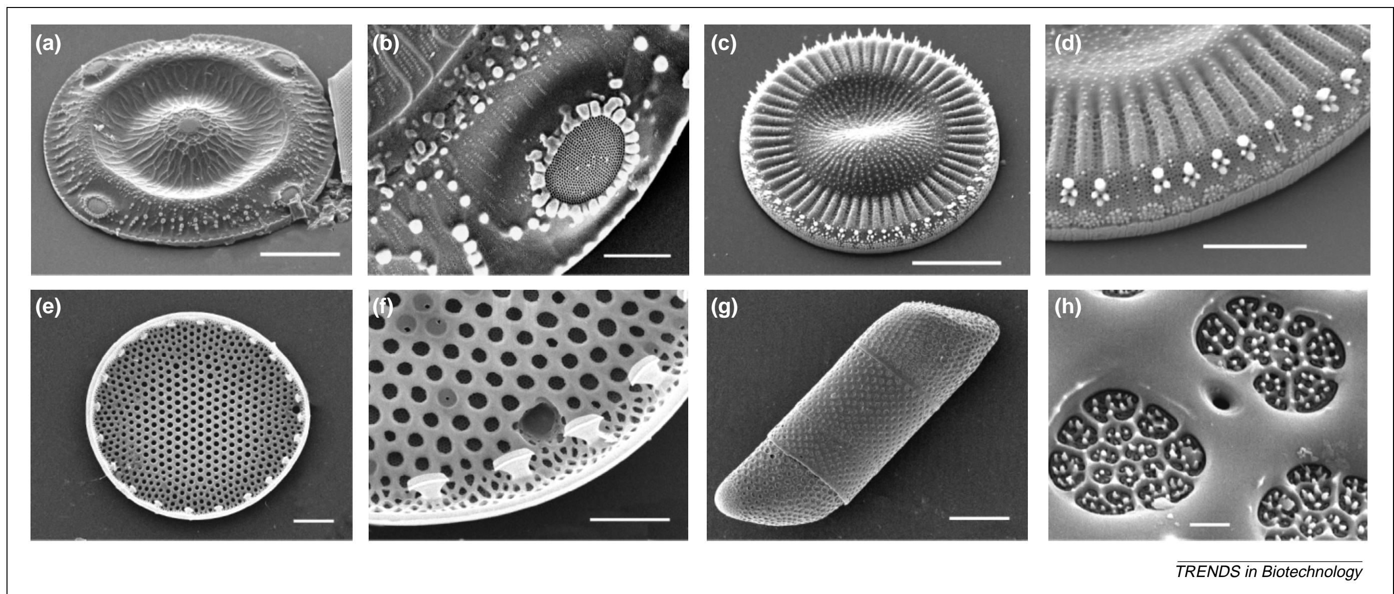

Fig. 2. Pairs of scanning electron micrographs (SEMs) showing marine centric diatoms and close-ups of surface features, courtesy of Mary Ann Tiffany. (a,b) *Glyphodiscus stellatus*, a very rare diatom from Mission Bay, San Diego, CA, USA (taken June 11, 2002); scale bar on the whole valve is 20 μm, on the close-up is 5 μm. (c,d) *Cyclotella meneghiniana* with asymmetric central buckling; scale bar 20 μm, close-up at 5 μm. For (c), radially oriented microtubules might provide a 'prepattern' for diffusion limited aggregation [41], a compromise between the two morphogenesis hypotheses. (e,f) *Roperia tesselata* taken 5 km off San Diego; the first scale bar is 10 μm and the scale bar on the close up is 5 μm. (g,h) *Isthmia nervosa* from Providence Bay, Whidbey Island, Washington State, WA, USA) off red seaweed; scale bars are 50 μm and 1 μm.

thus a possible key to eukaryotic cell behavior. Given that diatoms move on surfaces, we could try to use patterned surfaces [11] to guide them into place, perhaps moved along by phototropism or geotropism [12,13], where they could become nanotechnology components.

In the remarkable colonial diatom *Bacillaria paradoxa* the cells move rhythmically against each other [1,14] (also A.P. Ussing *et al.*, in preparation), like a deck of cards sliding back and forth. (For an online movie see http://thalassa.gso.uri.edu/plankton/theater/alphabetical.htm.). Because the velocities are additive, this is the world's fastest microorganism.

Diatom morphogenesis

Diatom shells are composed of amorphous silica [15] but are highly structured. They are basically made of hydrated glass: $SiO_2.n\,H_2O$. There are 10^5-10^6 species of diatoms, distinguished by their shells [16]. How does an amorphous substance produce such a variety of organized shapes? Two major approaches have been followed: either the pattern forms spontaneously out of silica by diffusion limited aggregation (DLA) or there is a prepattern of something else onto which or within which the silica precipitates (which begs the question as to what causes that pattern) [17,18]. Neither approach has seriously invoked the genetics of diatoms, so there is a lot of work ahead. Although diatoms might not tell us how legs and arms and brains of vertebrates are put together, bridging the intellectual gap from the genome (now being sequenced in diatoms [19]) to diatom shell structure would be a great accomplishment. Intermediaries that have been discovered are the silica binding polyamines and silaffins [20]. Diatom DNA synthesis is silica dependent [21] and a silica membrane transport system exists [22]. Whether these molecular components merely concentrate silica and catalyze its (fractal) DLA precipitation, or somehow form a prepattern onto which silica precipitates, has yet to be determined. They probably account for the 20–40% organic content of diatom silica [17,18].

The big question, which is also fundamental to debates in evodevo (evolution and development), is whether diatoms (or any organisms) vary 'infinitely', or are subject to 'developmental constraints'. Fortunately, we can speed up evolution in the lab, especially for microorganisms, using mutagenic chemicals or ultraviolet light, and assorted methods for selecting those mutants that do best under conditions set. A classical device is the chemostat, which acts similarly to an artificial stomach for microorganisms to grow in: those that grow too slowly are washed out. It thus selects for speed of growth, amongst those mutants that can use the nutrients that are placed in the incoming medium. A variant of this idea is the compustat [23], in which a computer decides 'who shall live and who shall die'. The compustat was conceived as a means of pushing diatom morphology to match any preconception we might have. It would work by visually scanning all diatoms in a small growth chamber with a digital camera attached to a motorized microscope, and matching the observed patterns [24] against an ideal 'template'. Those diatoms that were furthest from the ideal would be destroyed with a laser or UV microbeam and the rest subjected to mutagenesis. Hopefully someone will soon build a compustat.

Diatom nanotechnology

Most fabrication techniques in nanotechnology involve planar lithographic approaches [25]. Three dimensional structures are built up plane by plane, and excruciating acrobatics are required to etch away undercuts and cavities. Diatoms build directly in 3D, which is why they are so attractive for nanotechnology [26], and of course,

with exponential growth in numbers (at one cell division per day), any number of parts we want could be made.

The first artificial diatom patterns were synthesized by 3D vapor deposition of silica by Max Schultze back in 1863 (his plates are reproduced in [17,18]), and the feat has only been partially attempted since [27]. If silica is 'merely' precipitated onto an already existing scaffold (prepattern), as some would hypothesize, then we ought to be able to construct artificial scaffolds for silica precipitation. The physical chemistry of unassisted silica precipitation is well understood [28].

The most remarkable invention in the new field of diatom nanotechnology is that akin to the replicator [29] in the science fiction television and movie series Star Trek. Diatom shells are placed in an atmosphere of magnesium gas at 900°C for 4 hr. Apparently an atom for atom substitution of magnesium for silicon occurs, with no change in 3D shape. Thus silicon oxide, a material of limited usefulness in nanotechnology, is transformed into magnesium oxide [30]. The authors list nine other 'shape preserving gas/solid reactions' that are thermodynamically favored, and should 'replicate' diatom silica shells into a variety of oxides. The list of uses envisaged includes microcapsules for medications, water filters (sieves), catalytic substrates, sensors, optical diffraction gratings and actuators, or templates for any of these.

As mentioned, diatoms have a peculiar, silica-dependent mechanism for DNA synthesis [21,31]. This permits silica starvation synchrony to be achieved [32], by growing the diatoms in Teflon beakers to prevent them from extracting silica from glass. If pennate diatoms were then killed in mid-formation of their shells, we could produce massive quantities of nanoscale combs, such as are used in comb activators [33]. These could be sorted by size using light scattering in a FACS machine (fluorescence activated cell sorter).

Other sources of nanoscale silica objects, although not in such great variety, are the silica bodies found in grasses (Fig. 1d) [34]. One could imagine crops such as wheat being 'genetically engineered' and harvested not only for their seed but also for the silica bodies in their straw. Other, more esoteric organisms that precipitate silica include the radiolarians [35], sponges [36] and other algae [37–39].

The future

Diatom nanotechnology, conceived as an industrial process, started only as a suggestion in 1988 [40]. It is now a highly interdisciplinary, fast moving area, possibly headed for making a major contribution to nanotechnology. Biology as a source of nanotechnology has a few major advantages: large numbers of components are available via the exponential reproduction of the organisms that produce them, and mutation permits us to selectively evolve organisms with the components we want. In the course of pushing organisms to manufacture what we want them to, we are also likely to learn much about whether or not evolution is constrained. Thus bionanotechnology, although driven as an industrial pursuit, might nevertheless make fundamental contributions to our understanding of life.

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Putting some spine into alternative splicing

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Spinal muscular atrophy is a neurodegenerative disease caused by mutations of the *SMN1* gene. The homologous *SMN2* gene is unable to complement *SMN1* because of a crucial mutation in an exonic splicing enhancer, leading to alternative splicing and exclusion of exon 7. Two recent papers show that the defect in splicing of exon 7 of *SMN2* is specifically corrected by small synthetic effectors. These new and specific approaches have potential in the treatment of diseases caused by defective splicing.

Spinal muscular atrophy (SMA) is a degenerative disease of the motor nuclei in the spinal cord anterior horn and the lower brainstem. SMA presents in childhood with muscle weakness and, at the more severe end of the scale, fatal respiratory failure. Mutations of the *Survival of motor neurone* (*SMN*) locus on chromosome 5q13 are responsible for the disease. In humans, this locus contains an inverted duplication containing the telomeric *SMN1* gene and the centromeric, highly homologous *SMN2* gene. In SMA, there are missense, nonsense and splicing mutations in *SMN1*, which disrupt the protein [1]. SMN protein is ubiquitously found in all cell types. It is localized in subnuclear complexes known as 'gems', which are involved in small nuclear ribonucleoprotein assembly and recycling [2]. These 'gems', which can be visualized using immunofluorescence staining with SMN antisera, are absent in cells isolated from patients with SMA.

In mice, total deficiency of SMN by knockout of the orthologue causes embryonic lethality. In SMA, there is only a partial deficiency of SMN protein – the mutation of *SMN1* is partially complemented by *SMN2*, which is also transcribed. *SMN2* differs from *SMN1* by five nucleotides, which are translationally silent [3]. A cytosine to thymidine mutation at position +6 in exon 7 causes alternative splicing, by disrupting an exonic splicing enhancer (ESE)

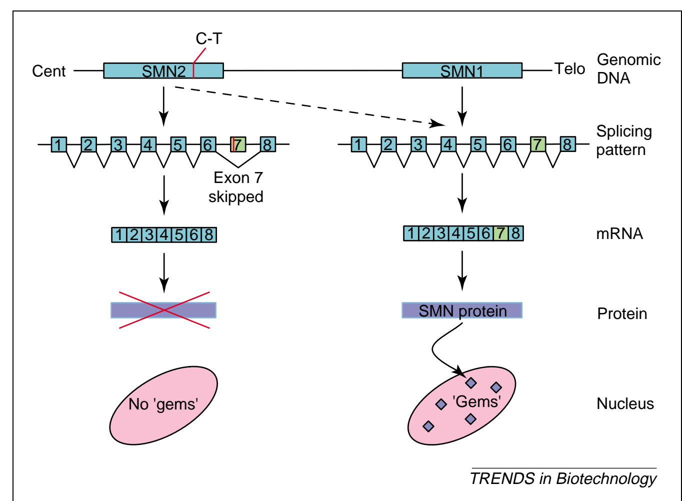

Fig. 1. Defective splicing in *SMN2*. The telomeric *SMN1* is spliced correctly with exon 7 included. Full-length SMN protein is generated, which is localised in subnuclear 'gems'. The centromeric *SMN2*, owing to the C-to-T mutation in exon 7 is spliced alternatively. A minority of *SMN2* transcripts, indicated by the dotted arrow, are spliced and translated to the full-length SMN protein. The majority of *SMN2* transcripts skip exon 7 to generate *SMN∆7*. The truncated product derived from the *SMN∆7* mRNA is unstable and is not incorporated into the 'gems'.

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