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MINI-REVIEW

Diatoms in biotechnology: modern tools and applications

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Abstract Diatoms have played a decisive role in the ecosystem for millions of years as one of the foremost set of oxygen synthesizers on earth and as one of the most important sources of biomass in oceans. Previously, diatoms have been almost exclusively limited to academic research with little consideration of their practical uses beyond the most rudimentary of applications. Efforts have been made to establish them as decisively useful in such commercial and industrial applications as the carbon neutral synthesis of fuels, pharmaceuticals, health foods, biomolecules, materials relevant to nanotechnology, and bioremediators of contaminated water. Progress in the technologies of diatom molecular biology such as genome projects from model organisms, as well as culturing conditions and photobioreactor efficiency, may be able to be combined in the near future to make diatoms a lucrative source of novel substances with widespread relevance.

Keywords Diatom · Nanotechnology · Biofuel · Bioremediation

Introduction

Approximately three billion years ago, photosynthetic bacteria brought about the oxygen synthesis that has since been sustained by some form of their progeny to which aerobic life owes its very existence to such organisms. Land-dwelling photoautotrophs were cultivated for agriculture and economic means early in human history, but it was only recently that global interest in marine algae was spawned by the realization that the ocean is a relatively untapped and unexplored source of biomass and novel biomolecules.

One set of major players in biomass production and the sinking of atmospheric greenhouse gas and biomass production are diatoms. Estimates indicate that these photosynthetically active organisms are responsible for 20–25% of total terrestrial primary production (Field et al. 1998) and approximately 40% of annual marine biomass production (Falkowski et al. 1998), making them the most dominant group of organisms sequestering carbon from the atmosphere. Diatoms are exceedingly robust and can inhabit virtually all photic zones from the equator to seemingly inhospitable sea ice where they are highly useful indicators of environmental conditions in their rapid response to environmental changes, including their capacity to react to sea ice freezing around them with their "natural antifreeze" ice-binding proteins (Janech et al. 2006). Thus, in all climate zones, diatoms show an exceedingly high degree of flexibility that could pose utilizations in biotechnological applications despite challenging conditions.

Along the same lines, diatoms have recently received further attention due to their enormous economic potential: Diatoms naturally produce substances for foodstuffs, antibiotics, and pharmaceutically active substances. This makes them a valuable resource for the acquisition of food supplements and substitutes for synthetic substances ranging from cosmetic chemicals to jet fuel. Industrial applications considered and initiated for commercial use of diatoms include nitrogen-fixing biofertilizer, renewable energy, fluid fuel production, raw materials production, and naturally-occurring and industrial waste detoxification using biological refuse as substrate. Furthermore, their

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unusual cell wall may prospectively be applied in nanotechnologies or computer chips.

Besides these important aspects, which have been reviewed recently (Kroth 2007), cell biological and molecular applications have been elaborated for diatoms in the last couple of years, inspiring enthusiastic perspectives for new applications of diatom biotechnology. In this field, diatoms may easily compete with other biological solar-powered factories, as cultivation needs light, water, and creativity only without the added drawback of vying with alimentary or bioethanol resources, since some diatoms readily thrive on such wastes as nutrients from sewage and carbon dioxide from gaseous exhausts—all without robbing food or cash crops of fertile soil.

In this paper, we introduce the cell biology of diatoms and summarize applications that are already available. Additionally, we review recent advances in techniques to manipulate diatoms, which can create genetically modified organisms with new capacities.

Evolution and cell biology of diatoms

Diatoms are unicellular phototrophs that evolved some 180 million years ago and may consist of more than 100,000 species (Drum and Gordon 2003; Kroth 2007), which have an ecologically significant role in biogeochemical cycling of carbon, phosphate, and silicon (Bidle et al. 2002; Falciatore and Bowler 2002; Lopez et al. 2005) as well as their massive reproduction and pollution of coastal waters in the form of blooms (Allen et al. 2005). Most diatoms are identified by their species-specific morphology of their amorphous silica cell wall, which vary from each other at the nanometer scale (Lopez et al. 2005). Although they seemingly contain the typical complement of eukaryotic compartments such as a cell nucleus, secretory machinery, and mitochondria at first glance, their plastidial ultrastructure has an increased complexity in that its stroma, the plasm of the plastid, is separated from the cytoplasm by four membranes and not by two as found in green or red algae and land plant plastids (Gibbs 1979). These additional membrane barriers seem to be indelible keepsakes derived from their cellular evolution (Cavalier-Smith 2000). Diatoms evolved via secondary endosymbiosis in which a heterotrophic host cell engulfed a unicellular phototroph with a plastid surrounded by two membranes (Maier et al. 2000). In order to optimize the symbiosis to make it most advantageous for the host, the physiology and biochemistry of the eukaryotic partners became intertwined, whereby the symbiont was reduced to minimized components within a plastid surrounded by four membranes. Reconstructing these events led to the identification of these individual membranes and the entailed luminal spaces separating them. The outermost membrane of the complex is the remaining phagotrophic vacuole and separates the former vacuole lumen from the host's cytosol (Hempel et al. 2007). This is followed by the former plasma membrane and remnant plasma of the eukaryotic symbiont (periplastidal compartment, PPC; Gould et al. 2008), while the inner two membranes are equivalent to the plastid envelope introduced into the cellular merging with the symbiont's plastid (Hempel et al. 2007). Thus, for biotechnological approaches, the complex membrane architecture of the plastid presents a challenge and an advantage—challenge on the one hand in targeting proteins, but an advantage for expressing proteins in sealed-off compartments like the PPC seen in Fig. 1, which are otherwise unavailable in many other algal kingdoms.

Presently available applications

Nutritional applications

High-quality vitamins, essential unsaturated fatty acids, amino acids, and food supplements are naturally synthesized by most algae including diatoms and comparable to conventional vegetable sources (Becker 2007). Prior limitations imposed upon mass production of such products from diatoms are mainly limited by production costs and palatability, resulting in such algal products presently being limited to such nutritional applications as health food, cosmetics, and animal feed.

The most nutritionally relevant biomolecules produced by diatoms are unsaturated fatty acids like eicosapentaenoic acid (EPA, 3.9–5% of dry weight in *Phaeodactylum tricornutum* (Lebeau and Robert 2003; Meiser et al. 2004), arachidonic acid (Lebeau and Robert 2003), docosahexaenoic acid (Kroth 2007), and other omega-3 fatty

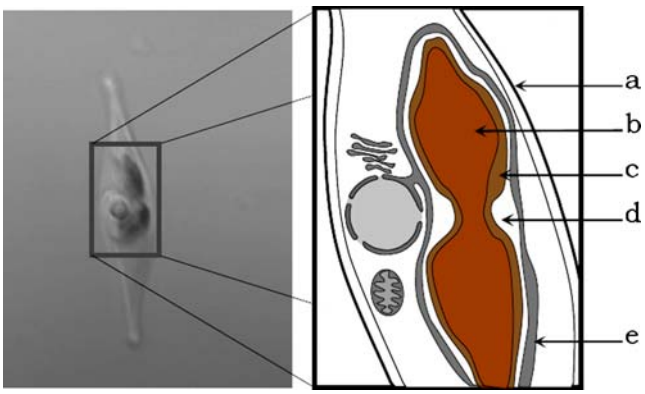

Fig. 1 Plastid compartmentalization in *P. tricornutum* resulting from its evolution as a diatom. **a** Silica cell wall, **b** stroma, **c** intermembrane space, **d** periplastidal compartment, and **e** chloroplast ER. Light microscopy courtesy of F. Hempel; schematic drawing modified from M. Sommer

acids (Sheehan et al. 1998). They reduce cardiovascular disease (Lebeau and Robert 2003), are precursors for important tissue hormones, are present in 20% of the brain's grey matter (Kroth 2007), and are generally thought to be anticarcinogenic. Optimal photoautotrophic conditions lead to an average productivity of 530 mg omega-3-fatty acid and 118 mg EPA per liter per day in *P. tricornutum* (Meiser et al. 2004), whereby optimized heterotrophic growth of *Nitzschia laevis* yielded 175 mg EPA per liter per day (Becker 2007; Wen and Chen 2001). Due to its abundance (up to 30% of all fatty acids) and constituting the overwhelming majority of the polyunsaturated fatty acids in diatoms, purification of EPA from diatoms is inexpensive in contrast to fish liver oil, the other natural source, without the disadvantage of peculiar taste, instability, and high purification costs (Lebeau and Robert 2003).

Industrial applications

Algal biomass contains the following three main components and fuel production possibilities: carbohydrates for ethanol production via fermentation, proteins for methane production via anaerobic gasification, and natural oils for biodiesel production.

Fluid fuel synthesis

Diatoms store carbon in the form of natural oils. Cultivation of algae, e.g. diatoms, with a lipid content of 70% dry weight would require a surface of 4 MHa—2.2% of arable land in the USA—in a zone of high sun exposure to totally replace petroleum consumption in the USA (Chisti 2007). Besides industrial exhaust, biological refuse such as animal waste water and sewage can be used to feed diatoms due to their enriched urea and nutrient content that can stimulate algal growth at the correct concentrations. Production costs are presently estimated at 2.80 USD/liter algal biodiesel assuming 30% oil content (Chisti 2007), which equates to approximately 2.8 times the price of oil and refinement for a liter posted by the US Energy Information Agency in summer 2008 (http://tonto.eia.doe.gov/oog/info/gdu/gasdiesel.asp). Nonetheless, because diatoms grow in aqueous suspension and have more efficient access to water, CO_2, and other nutrients, they can convert into lipid stocks of up to 85% of their total weight (Lebeau and Robert 2003) and can produce 30 times the amount oil per area of terrestrial oilseed crops. It has been estimated that 200 kHa of open diatom/green algae ponds could produce liquid fuel energy equivalent to 10^{15} BTUs (~10^{18} kJ) even without gene manipulation or optimization via photobioreactors (Sheehan et al. 1998).

Nanotechnology

Diatoms biomineralize a mixture of silica, proteins, and carbohydrates to form an intricately patterned inorganic silica shell that surpasses modern engineering capabilities. These frustules serve as a biomimetic model silica structures at the micro-, meso-, and nanoscale (Hildebrand et al. 2008; Lopez et al. 2005) and are presently commonly used as diatomaceous filters in applications ranging from filtering liquids to DNA purification (Gilmore et al. 1993) to adsorbing heavy metals (Al-Degs et al. 2001).

Probably, the most advantageous aspect of these silica nanostructures is that their synthesis occurs under ambient conditions: Amorphous silica are transported in vesicles to be precipitated on a carbohydrate and peptide scaffolding (Parkinson et al. 1999). The smoothing of the silica aggregate within the vesicles, or sintering, is apparently a process that is affected by pH and temperature in vivo (Parkinson et al. 1999). Thanks to recently sequenced diatom genomes (Armbrust et al. 2004; Kroth et al. 2008), the capacity to elucidate the nature and genetic basis of this interspecies variability will doubtlessly be forthcoming and bolster future manipulation and design of frustule nanostructures, which apparently varies significantly even within species (De Martino et al. 2007).

The ability to genetically engineer diatoms to synthesize designer frustules is anticipated to have wide range applications as microelectronic devices, chemical and biological sensing and diagnosis (Bismuto et al. 2008), as drug delivery systems (Wee et al. 2005), catalysis (Jia et al. 2007), highly efficient nanofiltration (Parkinson et al. 1999), and energy storage (Pérez-Caberoa et al. 2008), including as capacitors (Weatherspoon et al. 2005). These structures could also have potential for light-emitting display and optical storage (Parkinson et al. 1999). Furthermore, frustule silicate can be completely replaced on the atomic level with magnesium oxide, implicating frustules in nanometallurgy for "casting" metal nanostructures (Drum and Gordon 2003). In order to unravel the genetic and molecular components as well as the mechanisms involved in diatom silica frustule synthesis, great strides must be made in the fundamental research into this topic that have only recently commenced (Mock et al. 2007).

Phytoremediation of heavy metals contamination

When exposed to heavy metals, plants, algae, and some fungi synthesize oligomers of glutathione known as phytochelatins (Grill et al. 1985). Phytochelatins (PC) are intracellular and extracellular chelators, having the general structure of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, whereby n normally varies from 2 to 11 (Toppi and Gabbrielli 1999). Cumulatively, plants, animals, algae, and fungi PCs are induced by such

heavy metals from soil and water as Al, Ag, Cd, Co, Cr, Cs, Cu, Hg, Mg, Mn, Mo, Ni, Pb, Se, Sr, and Zn (Cobbett and Goldsbrough 2002; Delhaize and Ryan 1995; Prasad and Freitas 2003; Salt et al. 1995). PCs are therefore ecologically important for heavy metal detoxification both within cells and tissues as well as external chelation of metals using types of organic chelation.

Understanding the mechanistic details of chelator–substrate binding and how they differ from one another according to substrate specificity would make them available to industrial use via (over)expression in diatoms for the phytoremediation of contaminated sites (Salt et al. 1998). Intracellular PCs in metal-binding peptides are characterized by a high cysteine content in peptides and have already been studied in laboratory cultures of the marine diatom *P. tricornutum* exposed to Cd, Pb, or Zn. The prospect of discovering novel natural or artificial phytochelatins, cation diffusion facilitators, and metallothioneins would broaden the spectrum of metals available for transgenic applications (Prasad and Freitas 2003). Altogether, plant, algal, and fungal peptides, polypeptides, and proteins expressed in diatoms could possibly bind and collect metal contaminants in polluted waters as transgenic plants are not able to without soil. Furthermore, the optimization of metal, phosphate, and nitrogen recovery could be even used to reacquire raw materials from wastewater in addition to reducing the cost of wastewater purification (Lebeau and Robert 2003).

Bioproductivity of diatoms

The economic feasibility of industrial production of biomolecules by engineered organisms is dependent on cheap culturing. Like other photosynthetic organisms, diatoms require merely carbon dioxide, water, inorganic salts, and light to grow, so culturing media for candidates of interest would consist of readily available fresh, sea, or brackish water, thus making medium enrichment with organic compounds unnecessary (Lopez et al. 2005). Furthermore, diatom culturing would not compete for agricultural cropland, because containment and optimal growth depends on fermentors that could even be located in deserts (Gordon and Polle 2007). Optimal production efficiency of such bioreactors depends on the qualities of the light applied, which, in principle, is delivered naturally by the sun or artificially via photovoltaic technology.

In the 1970s, the United States Department of Energy sponsored the Aquatic Species Program in which it tested the viability of using algae species found in American waters in ponds at its Roswell, New Mexico test site. This program determined that the maximum recovery rates in these churned open-air ponds of 50 g dry algae per square meter per day could be harvested (Sheehan et al. 1998). To date, greater biomass yields are speculated with state-of-the-art fermentors and theoretical data to improve the yields significantly. A recent review of the subject estimated a theoretical recovery of over 100 g dry weight per square meter per day projected using pulsed LED photobioreactors (Gordon and Polle 2007), whereas another review describes the present practical maximum biomass production as being 38 g per square meter per day (Eriksen 2008). Other technologies for increasing photobioreactor efficiency include reducing photosynthetic antenna size to increase photosynthetic efficiencies (Mussgnug et al. 2007) as well as limiting the extent of photoreactions to specific wavelengths in order to diminish the saturation of the photosynthetic machinery and allow dark reactions to occur practically simultaneously (Gordon and Polle 2007).

Further advantages of biotechnology in diatoms

Among the artificially synthesized substances for use in medicine and pharmacy are antibodies, vaccines, hormones, and enzymes. These are currently produced in bacteria, yeast, and mammalian cell culture, which have the disadvantage of toxins, viruses, and expense. Plants have been used in the past to circumvent these disadvantages, but themselves have the added disadvantage of transgenic pollen, which possibly leads to undesired propagation of genetic modifications (Nigh et al. 2000; Wolfenbarger and Phifer 2000), in addition to competing for land plots normally allocated by the primary sector for agriculture. Diatoms have the added advantageous potential to compartmentalize the processes introduced by transgenetics, such that biochemical precursors could be synthesized in one compartment apart from other biochemical processes for subsequent processing in a separate compartment. Because some diatoms can be grown asexually and in closed fermentors, the problems associated with other expression systems would be avoided.

Diatom genetic modification and future prospects

Besides harvesting naturally occurring substances from diatoms grown optimally in bioreactors to high optical densities, the next important prerequisite for achieving lucrative production of substances in diatoms is gaining as much knowledge about the organisms as possible, which has been a continuing work-in-progress for many years (Round et al. 2000).

Milestones in broadening the understanding of diatom cell biology and biochemistry have been genome projects, which are, in some cases, already published or underway (http://genome.jgi-psf.org/Phatr2/Phatr2.home.html; Armbrust et al. 2004; Kroth et al. 2008). The complete data from *Thalassiosira pseudonana* and *P. tricornutum* are used for

reconstructing the cellular biochemistry and compartmentalization thereof in diatoms (Kroth et al. 2008; Mock et al. 2007). Localization assays with topogenic signals fused to GFP led to the realization that targeting to various compartments within organelles are highly differentiated (Apt et al. 1996, 2002; Gould et al. 2006a, b; Kilian and Kroth 2005, Sommer et al. 2007). This determination was recently confirmed by in depth in silico data showing that enzymes for biochemical pathways are not always localized to one compartment in diatoms (Kroth et al. 2008). This knowledge in turn provides an essential basis for genetically modifying diatoms to selectively compartmentalize novel biochemical capacities to particular cellular locations in which key precursors are already present and can be redirected into a desired biochemical pathway.

One compartment, which may prove to be a very lucrative site for introducing new and innovative capacities may be the PPC, the remnant cytoplasm of a eukaryotic endosymbiont, sealed off by the surrounding plastid membranes.

Transformation

For several diatoms, including the already sequenced *P. tricornutum* and *T. pseudonana*, transformation protocols were broadened to introduce genetic material into diatoms (Apt et al. 1996; Poulsen et al. 2006). All of these protocols are based on biolistic methods, which led to random but stable integration of foreign DNA into the chromosomes of the cell nucleus. In combination with bioinformatics, copious amounts of knowledge have been obtained by transformation studies in recent years that now allow for topogenic signal-mediated protein targeting to virtually any cellular compartment of interest (Apt et al. 2002; Gould et al. 2006a, b; Kilian and Kroth 2005; Lang et al. 1998). Thus, using this complement of techniques, diatoms are rife for synthetic biology. Nonetheless, a technique that would be very helpful for many applications would be the ability to supplant endogenous genes with introduced ones via homologous recombination. In order to accomplish such an aim, protocols must necessarily be optimized and the frequency of transformation increased.

A further restriction on transformation is that the cell nucleus is presently the exclusive target. Transforming the plastid genome directly would be especially useful in the expression of genes of prokaryotic origin because it would minimize gene design and permit transfer directly to optimal expression conditions. Overcoming such restrictions would first require progress in transformation efficiency at the least. Furthermore, such aims would probably necessitate novel selection markers not presently in use because only a handful of markers generating resistance to antibiotics have already been tested (Apt et al. 1996), summarized in Table 1. The use of a combination of multiple selection markers within one diatom could be an effective alternative to the present lack of antibiotic resistances (Poulsen et al. 2006).

A further prerequisite for effectively genetically engineering diatoms is the use of inducible promoters, some of

Table 1 Summary of published transformable organisms and molecular tools involved in genetic manipulation of diatoms

Organism	Promoter	Marker	Antibiotic	Reporter/expressed gene	Reference
Cyclotella cryptica	Acc	nptII	G418	–	1
Navicula saprophilia	acc	nptII	G418	–	1
Cylindrotheca fusiformis	fcp	sh-ble	Zeocin	eGFP	2, 3
	nr			frustulin	
	fruα			hexose-transporter	
Thalassiosira weissflogii	fcp	sh-ble	Phleomycin/zeocin	uidA	4
Thalassiosira pseudonana	fcp	nat1	Nourseothricin	eGFP	5
	nr	sh-ble	Zeocin[a]		
Phaeodactylum tricornutum	fcp	sh-ble nat1/sat-1 nptII	Zeocin nourseothricin	eGFP/eCFP/eYFP	4,6,7,8
	nr		G418[a]	uidA	
	ca			cat	
	PCMV			Luciferase	
	PCaMV35S				
	PRSV-LTR				

Table modified from Kroth (2007). References: 1—Dunahey et al. (1995), 2—Poulsen and Kroger (2005), 3—Fischer et al. (1999), 4—Falciatore et al. (1999), 5—Poulsen et al. (2006), 6—Apt et al. (1996), 7—Zaslavkaia et al. (2000), 8—Sakaue et al. (2008)

acc acetyl-CoA carboxylase, *fcp* Fucoxanthin chlorophyll a/c-binding protein, *nr* Nitratreductase, *fruα* frustulin α 2/3, *ca* carbonic anhydrase, *PCMV* promoter sequences of the cytomegalovirus, *PRSV-LTR* promoter of Rous sarcoma virus long terminal repeat, *PCaMV35s* promoter of cauliflower mosaic virus 35s, *Sh-ble* zeocin/bleomycin binding protein, *Nat1* nourseothricin acetyltransferase, *Sat-1* streptothricin acetyltransferase, *nptII* neomycin phosphotransferase II, *uidA* β-glucuronidase, *cat* chloramphenicol acetyl transferase

[a] Low efficiency

which having already become available. Overexpression using a light-regulated promoter is in common use, the disadvantages of which being the long (1–2 days) induction time for expression with this promoter as well as the intense light requirements (Apt et al. 1996). Induction of expression at high levels within minutes is shown to be possible with the promoter of the nitrate reductase, initially characterized in *Cylindrotheca fusiformis* (Poulsen and Kroger 2005) and then in *T. pseudonana* (Poulsen et al. 2006). Thus, introduction and expression of heterologous genes in diatoms seems to be a manageable task. Nonetheless, techniques for homologous recombination and for silencing of endogenous genes, i.e. siRNA and/or miRNA, must be developed.

Summary

The most apparent problem with using diatoms for industrial, commercial, and nutritional applications heretofore has been the cheapness of the raw material of fossil oil and the availability of easy processing of plant crops into cheap fuels via fermentation. Due to the tenfold increase in oil price over the last 10 years and boosted environmental awareness in the general public, interest in the need for increased carbon-neutral, renewable resource recovery has dramatically increased worldwide. As land area for fuel crops become increasingly scarce and competition for arable land for agriculture increases, algal applications seem to become increasingly enticing. The gap between the cost of fuel cost and algal fuel production seems to be coming to a close, and projections do not consider the immense amount of oil that diatoms can produce (using 30% instead of 85% of dry weight). Ideas as to funneling this carbon storage into the in vivo production of other materials with genetic engineering have not nearly reached fruition. Thus, future optimization of resource production via genetic manipulation is definitively the method of choice for making diatom biotechnology not just viable but lucrative.

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References

Al-Degs Y, Khraisheh MA, Tutunji MF (2001) Sorption of lead ions on diatomite and manganese oxides modified diatomite. Water Res 35:3724–3728

Allen JT, Brown L, Sanders R, Moore CM, Mustard A, Fielding S, Lucas M, Rixen M, Savidge G, Henson S, Mayor D (2005) Diatom carbon export enhanced by silicate upwelling in the northeast Atlantic. Nature 437:728–732

Apt KE, Kroth-Pancic PG, Grossman AR (1996) Stable nuclear transformation of the diatom *Phaeodactylum tricornutum*. Mol Gen Genet 252:572–579

Apt KE, Zaslavkaia L, Lippmeier JC, Lang M, Kilian O, Wetherbee R, Grossman AR, Kroth PG (2002) In vivo characterization of diatom multipartite plastid targeting signals. J Cell Sci 115:4061–4069

Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, Zhou S, Allen AE, Apt KE, Bechner M, Brzezinski MA, Chaal BK, Chiovitti A, Davis AK, Demarest MS, Detter JC, Glavina T, Goodstein D, Hadi MZ, Hellsten U, Hildebrand M, Jenkins BD, Jurka J, Kapitonov VV, Kroger N, Lau WW, Lane TW, Larimer FW, Lippmeier JC, Lucas S, Medina M, Montsant A, Obornik M, Parker MS, Palenik B, Pazour GJ, Richardson PM, Rynearson TA, Saito MA, Schwartz DC, Thamatrakoln K, Valentin K, Vardi A, Wilkerson FP, Rokhsar DS (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science 306:79–86

Becker EW (2007) Micro-algae as a source of protein. Biotechnol Adv 25:207–210

Bidle KD, Manganelli M, Azam F (2002) Regulation of oceanic silicon and carbon preservation by temperature control on bacteria. Science 298:1980–1984

Bismuto A, Setaro A, Maddalena P, Stefano LD, Stefano MD (2008) Marine diatoms as optical chemical sensors: a time-resolved study. Sens Actuators B, Chem 130:396–399

Cavalier-Smith T (2000) Membrane heredity and early chloroplast evolution. Trends Plant Sci 5:174–182

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

De Martino A, Meichenin A, Shi J, Pan K, Bowler C (2007) Genetic and phenotypic characterization of *Phaeodactylum tricornutum* (Bacillariophyceae) accessions. J Phycol 43:992–1009

Delhaize E, Ryan PR (1995) Aluminum toxicity and tolerance in plants. Plant Physiol 107:315–321

Drum RW, Gordon R (2003) Star trek replicators and diatom nanotechnology. Trends Biotechnol 21:325–328

Dunahey T, Jarvis E, Roessler P (1995) Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophila*. J Phycol 31:1004–1012

Eriksen NT (2008) The technology of microalgal culturing. Biotechnol Lett 30:1525–1536

Falciatore A, Bowler C (2002) Revealing the molecular secrets of marine diatoms. Annu Rev Plant Biol 53:109–130

Falciatore A, Casotti R, Leblanc C, Abrescia C, Bowler C (1999) Transformation of nonselectable reporter genes in marine diatoms. Mar Biotechnol (NY) 1:239–251

Falkowski PG, Barber RT, Smetacek VV (1998) Biogeochemical controls and feedbacks on ocean primary production. Science 281:200–207

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240

Fischer H, Robl I, Sumper M, Kröger N (1999) Targeting and covalent modification of cell wall and membrane proteins heterologously expressed in the diatom *Cylindrotheca fusiformis* (Bacillariophyceae). J Phycol 35:113–120

Gibbs SP (1979) The route of entry of cytoplasmically synthesized proteins into chloroplasts of algae possessing chloroplast ER. J Cell Sci 35:253–266

Gilmore S, Weston P, Thomson J (1993) A simple, rapid, inexpensive and widely applicable technique for purifying plant DNA. Aust Syst Bot 6:139–142

Gordon JM, Polle JE (2007) Ultrahigh bioproductivity from algae. Appl Microbiol Biotechnol 76:969–975

Gould SB, Sommer MS, Hadfi K, Zauner S, Kroth PG, Maier UG (2006a) Protein targeting into the complex plastid of cryptophytes. J Mol Evol 62:674–681

Gould SB, Sommer MS, Kroth PG, Gile GH, Keeling PJ, Maier UG (2006b) Nucleus-to-nucleus gene transfer and protein retargeting into a remnant cytoplasm of cryptophytes and diatoms. Mol Biol Evol 23:2413–2422

Gould SB, Waller RF, Mcfadden GI (2008) Plastid evolution. Annu Rev Plant Biol 59:491–517

Grill E, Winnacker EL, Zenk MH (1985) Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230:674–676

Hempel F, Bozarth A, Sommer MS, Zauner S, Przyborski JM, Maier UG (2007) Transport of nuclear-encoded proteins into secondarily evolved plastids. Biol Chem 388:899–906

Hildebrand M, Doktycz MJ, Allison DP (2008) Application of AFM in understanding biomineral formation in diatoms. Pflugers Arch 456:127–137

Janech MG, Krell A, Mock T, Kang J-S, Raymond JA (2006) Ice-binding proteins from sea ice diatoms (Bacillariophyceae). J Phycol 42:410–416

Jia Y, Hana W, Xionga G, Yanga W (2007) Diatomite as high performance and environmental friendly catalysts for phenol hydroxylation with H_2O_2. Sci Technol Adv Mater 8:106–109

Kilian O, Kroth PG (2005) Identification and characterization of a new conserved motif within the presequence of proteins targeted into complex diatom plastids. Plant J 41:175–183

Kroth P (2007) Molecular biology and the biotechnological potential of diatoms. Springer, Berlin

Kroth PG, Chiovitti A, Gruber A, Martin-Jezequel V, Mock T, Parker MS, Stanley MS, Kaplan A, Caron L, Weber T, Maheswari U, Armbrust EV, Bowler C (2008) A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis. PLoS One 3:e1426

Lang M, Apt KE, Kroth PG (1998) Protein transport into "complex" diatom plastids utilizes two different targeting signals. J Biol Chem 273:30973–30978

Lebeau T, Robert JM (2003) Diatom cultivation and biotechnologically relevant products. Part I: cultivation at various scales. Appl Microbiol Biotechnol 60:612–623

Lopez PJ, Descles J, Allen AE, Bowler C (2005) Prospects in diatom research. Curr Opin Biotechnol 16:180–186

Maier UG, Douglas SE, Cavalier-Smith T (2000) The nucleo morph genomes of cryptophytes and chlorarachniophytes. Protist 151:103–109

Meiser A, Schmid-Staiger U, Trösch W (2004) Optimization of eicosapentaenoic acid production by *Phaeodactylum tricornutum* in the flat panel airlift (FPA) reactor. J Appl Phycol 16:215–225

Mock T, Samanta P, Iverson V, Berthiaume C, Robison M, Holtermann K, Durkin C, Bondurant S, Richmond K, Rodesch M, Kallas T, Huttlin E, Cerrina F, Sussman M, Armbrust EV (2007) Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bioprocesses. PNAS 105:1579–1584

Mussgnug JH, Thomas-Hall S, Rupprecht J, Foo A, Klassen V, Mcdowall A, Schenk PM, Kruse O, Hankamer B (2007) Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. Plant Biotechnol J 5:802–814

Nigh R, Benbrook C, Brush S, Garcia-Barrios L, Ortega-Paczka R, Perales HR (2000) Transgenic crops: a cautionary tale. Science 287:1927

Parkinson J, Brechet Y, Gordon R (1999) Centric diatom morphogenesis: a model based on a DLA algorithm investigating the potential role of microtubules. Biochim Biophys Acta 1452:89–102

Pérez-Caberoa M, Puchola V, Beltrána D, Amorós P (2008) *Thalassiosira pseudonana* diatom as biotemplate to produce a macroporous ordered carbon-rich material. Carbon 46:297–304

Poulsen N, Kroger N (2005) A new molecular tool for transgenic diatoms: control of mRNA and protein biosynthesis by an inducible promoter-terminator cassette. FEBS J 272:3413–3423

Poulsen N, Chesley P, Kröger N (2006) Molecular genetic manipulation of the diatom *Thalassiosira pseudonana* (Bacillariophyceae). J Phycol 42:1059–1065

Prasad M, Freitas H (2003) Metal hyperaccumulation in plants—biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 93:285–321

Round FE, Crawford RM, Mann DG (2000) Diatoms: biology and morphology of the genera. Cambridge University Press, Cambridge, England

Sakaue K, Harada H, Matsuda Y (2008) Development of gene expression system in a marine diatom using viral promoters of a wide variety of origin. Physiol Plant 133:59–67

Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology (NY) 13:468–474

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668

Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of energy's aquatic species program: biodiesel from algae

Sommer MS, Gould SB, Lehmann P, Gruber A, Przyborski JM, Maier UG (2007) Der1-mediated preprotein import into the periplastid compartment of chromalveolates? Mol Biol Evol 24:918–928

Toppi LSD, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Weatherspoon MR, Allan SM, Hunt E, Cai Y, Sandhage KH (2005) Sol–gel synthesis on self-replicating single-cell scaffolds: applying complex chemistries to nature's 3-D nanostructured templates. Chem Commun 5:651–653

Wee KM, Rogers TN, Altan BS, Hackney SA, Hamm C (2005) Engineering and medical applications of diatoms. J Nanosci Nanotechnol 5:88–91

Wen ZY, Chen F (2001) A perfusion-cell bleeding culture strategy for enhancing the productivity of eicosapentaenoic acid by *Nitzschia laevis*. Appl Microbiol Biotechnol 57:316–322

Wolfenbarger LL, Phifer PR (2000) The ecological risks and benefits of genetically engineered plants. Science 290:2088–2093

Zaslavkaia L, Lippmeier C, Kroth P, Grossman A, Apt KE (2000) Transformation of the diatom *Phaeodactylum tricornutum* (Bacillariophyceae) with a variety of selectable marker and reporter genes. J Phycol 36:379–386