Silicification in the Microalgae

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1 Silicification in the Microalgae

Silicon (Si) is the second most common element in the Earth's crust (Williams 1981) and has been incorporated in species from most of the biological kingdoms (Knoll 2003). In this review I focus on what is known about: Si accumulation and the formation of siliceous structures in microalgae and some related non-photosynthetic groups, molecular and genetic mechanisms controlling silicification, and the potential costs and benefits associated with silicification in the microalgae. This chapter uses the terminology recommended by Simpson and Volcani (1981): Si refers to the element and when the form of siliceous compound is unknown, silicic acid, $Si(OH)_4$, refers to the dominant unionized form of Si in aqueous solution at pH 7–8, and amorphous hydrated polymerized Si is referred to as opal or silica.

2 Si Content across the Microalgae (and Some Related Groups)

Silicon has been found widely across the algae (Table 1). In some algal groups genetically controlled species-specific complex siliceous structures are formed (Fig. 1), while in other groups Si has been detected but has not been localized.

Silicon has been detected but poorly localized in the Cyanobacteria. Si has been detected both marine and freshwater genera of cyanobacteria, including species of *Synechococcus*, *Microcystis*, and *Spirulina/Arthrospira*, but the form of the Si and its location within the cell are unknown (El-Bestawy et al. 1996; Sigee and Levado 2000; Krivtsov et al. 2005; Baines et al. 2012). It has been hypothesized that, in some cases, the detected Si may be detrital and external to the cell wall, or Si may be bound to organic ligands associated with the glycocalyx, or that Si may accumulate in periplasmic spaces associated with the cell wall (Baines et al. 2012). In the case of field populations of marine *Synechococcus*, silicon to phosphorus ratios can approach values found in diatoms, and significant cellular concentrations of Si have been confirmed in some laboratory strains (Baines et al. 2012). The hypothesis that Si accumulates within the periplasmic space of the outer cell wall is supported by the observation that a silicon layer forms within invaginations of the cell membrane in *Bacillus cereus* spores (Hirota et al. 2010).

Significant quantities of Si, likely opal, have been detected in freshwater and marine green micro- and macro-algae (Fu et al. 2000), in particular the freshwater Hydrodictyaceae such as *Pediastrum* (Millington and Gawlik 1967; Sigee and Holland 1997), in some loricae of *Hemitoma* (Krienitz et al. 1990) and in the marine chlorophyte *Tetraselmis* (as *Platymonas*)[1] (Fuhrman et al. 1978). Si has been shown to be associated with the outer cell wall of *Pediastrum* and *Pedinomonas tuberculata* (Millington and Gawlik 1967; Preisig 1994). In the case of *P. tuberculata* Si is a component of the tubercular excrescence of the outer wall in the form of quartz (Manton and Parke 1960). Small amounts of Si have been detected widely across the Phaeophyceae (macroalgae) and in *Ptilonia okadai* (Rhodophyta) (Parker 1969; Fu et al. 2000; Mizuta and Yasui 2012). In *Saccharina japonica* Si is localized to the cuticle and mucilage caps of sori, in wounded tissues, and between the epidermal cells and outer cortical cells of sporophyte vegetative tissues (Mizuta and Yasui 2012). The Xanthophyceae may have silicified walls and cysts (Bold and Wynne 1978; Tappan 1980; Preisig 1994), but this should be confirmed (see Ariztia et al. 1991). Although rare, *Urceolus sabulosus* (Euglenophyta) is known

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[1]Wherever possible the currently accepted names for species are used. The name used in the paper cited is also indicated. For details of names see chapter "Systematics, Taxonomy and Species Names: Do They Matter?" of this book (Borowitzka 2016).

Table 1 Microalgae and related groups that accumulate silicon

Kingdom	Phylum, Class, Order (common name)	Example genera	Form of Si	Marine or fresh
Bacteria	Cyanophyta (Cyanobacteria)	*Synechococcus, Microcystis, Arthrospira*	Amorphous opal? Not localized	Marine and fresh
Plantae	Chlorophyta, Chlorophyceae (Green algae)	*Tetraselmis Pediastrum, Hydrodictyon*	Si in cell wall	Marine and fresh
Chromista	Cercozoa, Imbricatea (Filoseans)	*Euglypha, Thaumatomastix*	External scales	Marine and fresh
Chromista	Dinophyta, Dinophyceae (Dinoflagellates)	1. *Actiniscus*	1. Internal skeletal elements	Marine and fresh
		2. *Ceratium, Peridinium,*	2. Si in outer layer of cyst wall,	
		3. *Eodinia, Jusella*	3. Fossil genera with silicified cysts or theca	
Chromista	Haptophyta, Coccolithophyceae (Coccolithophores)	*Prymnesium neolepis*	External scales	Marine
Chromista	Ochrophyta, Chrysophyceae	*Chrysosphaerella, Paraphysomonas, Spiniferomonas*	Silicified cysts and some species have ornamented external scales in motile stage	Fresh and marine
Chromista	Ochrophyta, Synurophyceae	*Synura, Mallomonas*	All species have external scales in motile stage	Fresh
Chromista	Ochrophyta, Dictyochophyceae (Silicoflagellates)	*Dictyocha*	Siliceous skeleton of hollow rods	Marine
Chromista	Ochrophyta, Phaeophyceae (Brown algae)	*Ectocarpus, Macrocystis, Pelagophycus*	Not localized	Marine
Chromista	Ochrophyta, Bolidophyceae, Parmales	*Triparma, Tetraparma, Pentalamina*	External Si cell wall of interlocking plates	Marine
Chromista	Ochrophyta, Bacillariophyceae, Coscinodiscophyceae, Fragilariophyceae (Diatoms)	*Coscinodiscus, Chaetoceros, Nitzschia, Thalassiosira*, etc.	External 2-part ornamented skeleton, termed a frustule	Marine and fresh
Chromista	Ochrophyta, Xanthophyceae	*Acanthochloris bacillifera*	Si in cell wall and/or cysts	Fresh
Protozoa	Protozoa incertae sedis, Ebriophyceae (Ebridians)	*Ebridia, Hermesinum* (*Synechococcus*-like symbionts)	Internal skeleton of rugose or spiny rods	Marine

AlgaeBase was used for taxonomic classification
Guiry and Guiry (2015)

to accrete siliceous particles on their cell surface (Preisig 1994). Although currently the general consensus is that silicon is not a required nutrient for the groups discussed above, there is some evidence that Si deficiency and germanium dioxide reduce growth in some species of Chlorophyceae and Phaeophyceae (Moore and Traquair 1976; Tatewaki and Mizuno 1979; Mizuta and Yasui 2012). It has been hypothesized that Si accumulation may improve the resistance of the cell wall to decay or grazing or parasites, help with cell wall wound recovery, or that removal of Si from the environment may provide them with a competitive advantage against Si-requiring organisms such as the diatoms (Millington and Gawlik 1967; Fuhrman et al. 1978; Mizuta and Yasui 2012). Si has been shown to be bound to polysaccharide matrices as part of certain glycosaminogylcans and polyuronides (Schwarz 1973), providing support to the hypothesis that Si

may provide additional integrity to the cell wall, perhaps reducing decay and susceptibility to grazing and infection.

Complex siliceous structures, including intricate skeletons and scales, are formed by several groups within the Ochrophyta (Fig. 1a–h). The diatoms and the Parmales, both closely related to the naked flagellated Bolidophyceae (Daugbjerg and Guillou 2001; Lovejoy et al. 2006; Ichinomiya et al. 2011), produce external siliceous walls or plates that completely surround the plasmalemma. The diatoms produce a covering made of Si and tightly bound carbohydrates and proteins. This cell covering is composed of two siliceous valves (epi- and hypovalve) that fit together like a petri dish around the cell and are held together by overlapping siliceous girdle bands, and is termed a frustule (Fig. 1a). New daughter cells form within the parent frustule, causing mean population cell size to decrease with each round of asexual repro-
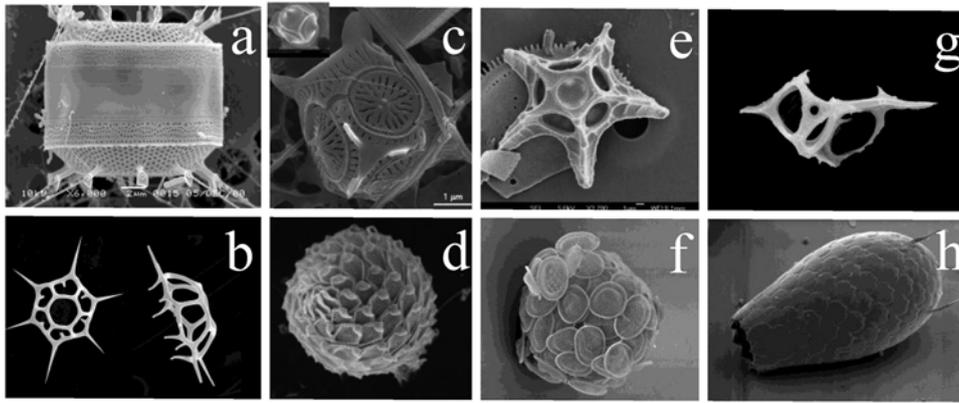
Fig. 1 Siliceous structures produced by microalgae and related groups. (**a**) Diatom frustule of *Thalassiosira* sp (Courtesy of Jim Ehrman and Mount Allison's Digital Microscopy Facility (MtA DMF)), (**b**) Silicoflagellate skeleton *Dictyocha* sp (courtesy of Jim Ehrman and MtA DMF), (**c**) Scales covering the Parmales *Tetraparma pelagica* (main panel, Courtesy of Drs. Susumu Konno and Richard W Jordan) and strain NIES 2565 (*inset*, Courtesy of Drs. Akira Kuwata and Mutsuo Ichinomiya), (**d**) Scale case of Synurophyta *Mallomonas* cf. *crassisiquama* (Courtesy of Astrid Saugestad), (**e**) Siliceous pentaster of the dinoflagellate *Actiniscus pentasterias* (Courtesy of Gloria Ernestina Sànchez with thanks to Drs. Diana Sarno and Marina Montressor), (**f**) Silicosphere of Haptophyte *Prymnesium/Hyalolithus neolithus* (Courtesy Dr. Masaki Yoshida), (**g**) Internal skeleton of Ebridian *Hermesinum adriaticum* (Courtesy of Dr. Paul Hargraves GSO-URI), (**h**) Scale test of Imbricatea *Euglypha* sp (By ja:User:NEON/commons:User:NEON_ja, Wikimedia Commons. Note: **a–f**, **h** are Chromista, **a–d** are Ochrophyta)

duction until a minimum threshold is met, triggering sexual reproduction. Maximum size is restored after sexual reproduction (Round et al. 1990). Valve shape, patterns of pores on the valve face, and structural features on the valves are used as taxonomic characters. At present the diatoms are the only known group with species with an absolute requirement for silicon, with the exception that *Phaeodactylum tricornutum* can grow without a silicified frustule, (Nelson et al. 1984) but always has lightly silicified girdle regions (Borowitzka and Volcani 1978). For most diatoms the cell division cycle becomes arrested at G1/S or G2/M without sufficient Si (Brzezinski et al. 1990; Brzezinski 1992).

The Parmales are pigmented, 2–5 μm diameter cells, surrounded by five or eight siliceous plates that fit together from edge to edge (Fig. 1c). The siliceous plates are ~80 nm thick and can have coarse or fine areolae, papillae, ornamented wings, keels and spines that can have very long projections (Booth and Marchant 1987; Ichinomiya et al. 2011; Konno and Jordan 2012). The cells are pigmented, contain chlorophylls *a* and *c*, have a large vacuole, and are not flagellated. Three genera and approximately 20 species have been described (Konno and Jordan 2012) with the number and characteristics of the plates used as taxonomic markers. At present silicification has been poorly characterized in the Parmales; only a single strain (NIES-2565) has been isolated and grown in culture and deposited in a culture collection (Ichinomiya et al. 2011). Although little is known about their biology, ecology and biogeographic distribution they are widespread and have been found in high numbers in polar and subpolar waters (Guillou 2011) and more rarely in tropical waters. More experimental work is required to determine if the Parmales have a strict growth requirement for Si and how they acquire Si and produce their siliceous plates.

There are two Phyla and three classes of microalgae that produce endogenous siliceous scales that surround the cell, the Chrysophyceae and the closely related Synurophyceae (Ochrophyta) and a few species within the Haptophyta. The Synurophyceae and Chrysophyceae include pigmented, chlorophylls *a* and *c*, fucoxanthin and violaxanthin, anthaxanthin and neoxanthin, solitary or colonial flagellated cells (Anderson 1987; Adl et al. 2012). All Synurophyceae and a number of species within the Chrysophyceae are covered in finely ornamented siliceous scales (Fig. 1d) and form siliceous cysts referred to as stomatocysts or statocysts (Sandgren et al. 1995). Some chrysophytes have siliceous loricae or outer coverings of basket-like siliceous rods (Tappan 1980). The siliceous scale-forming Chrysophyceae and Synurophyceae species typically have between one to four types of scales that are arranged on the outer surface of the protoplast. The scales can have complex ornamentation, including bristles and spines, and the total Si per cell can approach values reported for similarly sized diatoms (Leadbeater and Barker 1995; Sandgren et al. 1995). The Chrysophyceae and Synurophyceae examined do not have an absolute requirement for Si and can exist as naked (lacking siliceous scales) forms (Sandgren et al. 1996). When silicic acid is resupplied to cultures of cells without scales, growth rate is depressed until the siliceous scales are restored (Sandgren et al. 1996). Siliceous statocysts are found in freshwater and marine environments, are typically 3–35 μm in diameter, are flask or bottle shaped with a single pore with an organic plug that can be surrounded by a siliceous collar,

with a siliceous wall that can be smooth or reticulated and highly ornamented with spines, ridges, and punctae (Tappan 1980; Preisig 1994). Modern statocysts are common in freshwaters but fossil statocysts, sometimes referred to as Archeomonads, are more common in marine environments (Tappan 1980).

Most biomineralized scale-forming Haptophyta are calcareous but recently a marine haptophyte, *Hyalolithus neolepis* (=*Prymnesium neolepis*), covered with siliceous scales was characterized (Yoshida et al. 2006). *Prymnesium neolepis* has several layers of oval and hat-shaped siliceous scales (termed liths), 4–6 μm wide by 5–7 μm long, with a hyaline brim, and an elevated region perforated by many pores (Fig. 1f). Other species of *Prymnesium* have been characterized with cysts with siliceous material on the distal surfaces of their outermost scales (Green et al. 1982). Siliceous microfossils, identified as *Pseudorocella barbadiensis* Deflandre, 1938 by Perch-Nielsen (1978) from the late Eocene to early Oligocene (Perch-Nielsen 1978, Plate 7) bear some resemblance to the liths formed by *Prymnesium neolepis*, suggesting these, or related genera, may have a fossil record. It is worth noting that several different types of siliceous fossils have been assigned to *Pseudorocella*, so most observations of *Pseudorocella* in the fossil record do not resemble a *Hyalolithus*-type lith. Silicification and the Si requirement of *Hyalolithus*/*Prymnesium neolepis* have yet to be fully characterized.

The silicoflagellates, members of the Ochrophyta, Dictyochophyceae, have an amorphous siliceous skeleton of fused, tubular/hollow rods (Lipps 1970). There has been some disagreement about whether the skeleton is fully internal or external to the cell but the work of Moestrup and Thomsen (1990) strongly indicate it is external with the flagellated cell often lying in the cavity of the one-sided skeleton, although there is microscopic evidence that the cell may sometimes cover the outside of the other side of the skeleton (Scott and Marchant 2005). The skeletons of the modern genera *Dictyocha* are star-shaped, characterized by two different hexagonal rings interconnected by six bars that attach to the corners of the smaller ring to the mid-points of the sides of the larger ring, with spines projecting from the apices of the outer hexagon (Fig. 1b). Silicoflagellate taxonomy uses skeletal morphology to differentiate amongst taxa, although the very limited experimental work indicates there is significant phenotypic plasticity in the skeletons produced within single species of *Dictyocha* (Van Valkenburg and Norris 1970). Silicoflagellates have life cycle stages that lack a siliceous skeleton and the National Center for Marine Algae and Microbiota (NCMA) maintains a naked strain of *Dictyocha speculum* in culture, indicating that silicon is not an absolute requirement for the growth of silicoflagellates.

There is evidence of silicification within several orders within the Dinophyta. Species within the class Actiniscaceae,

a small, poorly studied group of heterotrophic Dinophyceae, are known to form internal biomineralized siliceous elements. Within *Actiniscus pentastarias* var. *arcticus*, 1–8 large and up to 14 smaller rudimentary star-shaped siliceous structures (pentasters) have been observed (Fig. 1e), although twin perinuclear penasters that surround the nucleus are most common (Bursa 1969; Hansen 1993; Preisig 1994). The form of Si is unknown although it has been assumed to be amorphous hydrated opal similar to what is formed by the diatoms. Bursa (1969) noted that for the Arctic lake species *A. pentastarias* v. *arcticus*, higher rates of silicification were associated with prolonged subzero temperatures and low light. A few other Dinophyta genera are reported to incorporate Si in their cell wall or cysts. High levels of Si have been detected in freshwater field populations of *Ceratium hirundinella*, Gonyaulacales (Sigee et al. 1999). Chapman et al. (1982) found that *C. hirundinella* produces two types of cysts: smooth-walled and granular walled cysts that are more resistant to decay. The young granular walled cysts contain vesicles filled with uniform electron dense granules ~60 nm in size (which could be silica nanospheres within a silicon deposition vesicle, see sections below) and an outer wall high in Si. There is also a report that *Peridinium cinctum* (Peridinales) may also form siliceous cysts (Eren 1969). A few siliceous fossil dinoflagellate theca and cysts have been described, *Eodinia* and *Lithodinia* with silicified walls from the Jurassic and siliceous cysts of *Lithoperidinium* from the Eocene and *Jusella* from the lower Oligocene (Loeblich and Loeblich 1984). There is some question about whether silicification in these rare specimens occurred before or after fossilization (Tappan 1980; Loeblich and Loeblich 1984). The Ebridophyceae, once thought to be Dinophyceae, but currently placed in their own class within the Protozoa *incertae sedis*, have an internal solid siliceous skeleton of rugose or spiny rods that are triaxially or tetraxially arranged (Fig. 1g). There are two extant species of Ebridians, *Ebridia tripartitia* and *Hermesinum adriaticum*. *Ebridia* is known to feed on diatoms and *Hermesinum* has *Synechococcus*-like endosymbiotic cyanobacteria (Hargraves 2002). Ebridians have never been cultured and little is known about how they acquire Si or form their siliceous skeletons. It would be interesting to determine how they acquire Si from the environment, if the cyanobacterial endosymbionts are involved, or if they recycle Si from diatom prey, or actively take up silicic acid from the environment.

Within the Cercozoa, the Imbricatea orders Euglyphida and Thaumatomonadida include heterotrophic species that form siliceous scales. In the past these groups have been referred to as Silicofilosea due to their external secreted siliceous scales and tubular cristae (Adl et al. 2012) but sequence analyses of rRNA and rDNA indicate they form separate groups within the Cercozoa (Wylezich et al. 2007; Ota et al. 2012). The Euglyphida and Thaumatomonadida have been

found in a wide variety of aquatic and marine habitats, but are common constituents of freshwater and estuarine benthic environments (Wylezich et al. 2007; Ota et al. 2012). Many species from these groups can produce solid or perforated external siliceous scales that surround the cell, with an opening for the cell to interact with the environment (Fig. 1h). The Euglyphida are testate filose amoebae (Ota et al. 2012). Many Euglyphida species have rounded to elliptical secreted siliceous plates that are bound by organic cement and when deprived of silicon in their media will produce deformed tests, cease to grow, and enter a quiescent state (Anderson 1990; Anderson and Cowling 1994). The Thaumatomonadida are gliding or swimming flagellated cells that can produce filopodia (Wylezich et al. 2007; Adl et al. 2012; Ota et al. 2012) and have oval or triangular siliceous scales often formed from the partial fusion of two plates that cover the body and sometimes the flagella. Siliceous fossil scales and body cases similar in morphology to the Euglyphida from the Lower Cambrian indicate that silicification in the Chromista may have very early origins (Allison 1981; Porter and Knoll 2009; Porter 2011).

3 Silicification: Molecular and Genetic Mechanisms

Most of what is known about silicification comes from work on diatoms, with some work on the Chrysophyceae and Synurophyceae and scattered observations on a few species from other classes of microalgae. There is very little information on how the other microalgae groups, even groups with complex siliceous structures, such as the silicoflagellates, Ebridia or Haptophyta, form their siliceous skeletons and scales.

3.1 Silicic Acid Transporters

Diatoms acquire silicic acid from the environment through diffusion and active uptake by silicic acid transporters (SITs) during cell wall formation in the synthesis phase of the cell division cycle (Hildebrand et al. 1997; Hildebrand 2003). The SITs belong to a unique gene family that differs fundamentally from silicon transporters identified from sponges and higher plants. To date SITs have been found in diatoms, Synurophyceae (*Synura petersenii*), Chrysophyceae (*Ochromonas ovalis*) and Choanoflagellates (Protozoa) with siliceous loricae (Hildebrand et al. 1997; Likhoshway et al. 2006; Sapriel et al. 2009; Marron et al. 2013). Currently it is unknown if SITs or other silicon specific transporters are present in other groups of silicifying micro- and macro-algae. Phylogenetic analysis indicates strong homology in the SITs analyzed from diatoms and the closely related

Chrysophyceae and Synurophyceae; most of the charged lysines and arginines are in the same positions (Likhoshway et al. 2006; Marron et al. 2013). The choanoflagellate SITs form a separate monophyletic clade from the diatoms, Chrysophyceae and Synurophyceae. Marron et al. (2013) hypothesize that the SITs in the choanoflagellates may have originated from horizontal gene transfer from the Ochrophyta. Fossil evidence indicates silicification in the Chromista goes back to the Neoproterozoic with the Euglyphida (Porter et al. 2003; Porter and Knoll 2009; Porter 2011) and perhaps the Chrysophyceae (Allison 1981; Allison and Hilgert 1986; Knoll 1992), suggesting that a heterotrophic Chromist ancestor or photosynthetic Chrysophyte may be the source for SITs in the diatoms. A clearer picture of the evolution of SITs across the microalgae will develop as more sequence data becomes available representing more species from more taxonomic groups.

The SITs from diatoms have ~550 amino acids with ten trans-membrane alpha helices and a carboxy terminal. Many of the SIT sequences examined have a putative sodium binding site and conserved GXQ, MXD (Curnow et al. 2012) and CMLD/MMLD motifs (Grachev et al. 2005; Sherbakova et al. 2005). The GXQ and MXD motifs are hypothesized to be involved in the binding of silicic acid (Curnow et al. 2012) and the CMLD/MMLD domain has been hypothesized to act as a binding site that plays a role in the formation of a channel for silicic acid transport (Sherbakova et al. 2005; Annenkov et al. 2013). At present, SITs have been observed in the plasmalemma and associated with the membranes of small and large intracellular vesicles (Sapriel et al. 2009), likely silica deposition vesicles.

Many of the diatom species examined have more than one SIT gene that may have originated from duplication events; SITs within species usually cluster more closely together than SITs across species (Thamatrakoln and Hildebrand 2005; Sapriel et al. 2009)). In *Thalassiosira pseudonana*, Tp*SIT1* and Tp*SIT2* have 95 % similarity in amino acid sequence, but Tp*SIT3* is only 74 % and 76 % similar to Tp*SIT1* and Tp*SIT2*, respectively (Thamatrakoln and Hildebrand 2007) and may have originated prior to the origin of the Thalassiosirales (Alverson 2007). SIT concentration and expression is often not well correlated with mRNA levels (Thamatrakoln and Hildebrand 2007). Phylogenetic analyses indicate a sequence in SIT trans-membrane region seven to eight may separate pennate species from most of the centric species (Thamatrakoln et al. 2006; Alverson 2007; Sapriel et al. 2009). It has been hypothesized that sequence differences across SITs may reflect: (i) differences in function, some SITs may be higher or lower affinity or capacity or be targeted to function during different parts of the cell cycle, or act as sensors of silicic acid concentration, or (ii) different localizations within the cell such as the plasmalemma or intracellular vesicles such as the SDV or (iii) dif-

ferences associated with function in fresh versus marine waters (Thamatrakoln and Hildebrand 2005; Thamatrakoln et al. 2006; Alverson 2007). Much more work is required to quantify how different SITs, across different species, respond to different environmental conditions.

3.2 Silica Deposition Vesicle

Diatom valves, and siliceous structures formed by most of the microalgae examined, are formed within acidic membrane-bound organelles termed silica deposition vesicles (SDVs). SITs have been observed on cellular vesicles with high silicic acid content (Sapriel et al. 2009) but it has yet to be determined if SITs supply the majority of the silicic acid required to fuel frustule formation (Vrieling et al. 1999). To date our understanding of the role of the SDV in silicification has been hampered by the inability to isolate and study the SDV directly. Most evidence suggests that the SDV most likely originates from the Golgi/endoplasmic reticulum network (Li and Volcani 1984). Smaller transport vesicles may provide substrate to the SDV including silicic acid, plasma membrane, and silica-forming peptides (Li and Volcani 1984; Vrieling et al. 2007; Sapriel et al. 2009; Annenkov et al. 2013). In diatoms the SDV develops inside the plasma membrane of the newly formed daughter cells along the cleavage furrow (Li and Volcani 1984). A separate SDV may form for the valve and girdle bands (Li and Volcani 1984). During valve formation the SDV rapidly expands, its movement controlled by the cytoskeleton (Tesson and Hildebrand 2010). Microscopic evidence indicates close associations between the SDV and different organelles across the major microalgae groups. For example, siliceous scales form within vesicles associated with Golgi bodies within the cytoplasm in the Euglyphida while the scales of Thaumatomonadida form within vesicles closely associated with the mitochondria (Ota et al. 2012).

Many of the detailed molecular mechanisms that control the complex structures that characterize diatom frustules still remain a mystery. It is clear that the SDV plays a key role in silicification. Polymerization of silicic acid into nanometer-sized structures, typically silica spheres, occurs within the SDV. It has been hypothesized that in situ silica precipitating peptides and organic scaffolds within the SDV and positioning and movement of the SDV by the cytoskeleton may control the development of the three-dimensional siliceous structures (Hildebrand 2003; Tesson and Hildebrand 2010). Microtubules position and strengthen the SDV and actin filaments are intimately associated with the development of micro-scale siliceous structures (Tesson and Hildebrand 2010). Once a base layer is established, silica-associated and silica-forming peptides may catalyze self-assembly of three-dimensional siliceous structures (see below). A network of

glucosamine has been found within the frustule wall of *T. pseudonana*, and chitin synthases are commonly present in diatoms, suggesting chitin may act as an organic scaffold for silicification or the strengthening of the frustule wall (Brunner et al. 2009; Durkin et al. 2009).

In the diatoms, Chrysophyceae, Synurophyceae, and Imbricatea, once siliceous structures are formed they are exocytosed (Ogden 1979; Li and Volcani 1984; Sandgren et al. 1996; Meisterfeld 2002). The siliceous scales of the Chrysophyceae, Synurophyceae, and Imbricatea are placed on the outside of the cell in species-specific arrangements. The formation of siliceous cysts within the Chrysophyceae and Synurophyceae is also associated with a silica deposition vesicle (Sandgren 1989). Patterns of silicification in the formation of the cyst wall varies across the species examined, but many species exhibit the formation of elongate finger-like Si projections, the production of irregular Si patches, or amorphous fine-grained Si accretion, followed by thickening and shaping of the cyst wall (Sandgren 1989). In the case of the internal siliceous pentasters of *Actiniscus pentasterias*, once the pentasters are formed the wall of the enclosing vesicle disintegrates (Bursa 1969).

3.3 Silica-Associated and Silica-Forming Peptides Found in the Diatom Frustule Wall

Several unique molecules have been isolated from diatom cell walls using anhydrous hydrofluoric acid or ammonium fluoride, including: frustulins, pleuralins, cingulins, silaffins, silacidins and long-chain polyamines. Frustulins are Ca^{2+} binding glycoproteins found on the outer protein coat of the frustule wall (Kröger et al. 1996), hypothesized to contribute to cell wall integrity (Poll et al. 1999). Pleuralins and cingulins are both localized in the girdle band region of the diatom frustule. The function of pleuralins is currently unknown but they are encoded by a small multi-gene family, are highly anionic, and tightly bound to diatom silica (Kröger and Wetherbee 2000; Zurzolo and Bowler 2001). Cingulins have been shown to be part of a chitin-independent organic matrix that becomes rapidly silicified in the presence of silicic acid (Scheffel et al. 2011) that are up regulated along with silaffin (*Tpsil1*) during cell wall and girdle band formation (Shrestha et al. 2012).

The most abundant molecules extracted from the diatom frustule are a diverse set of silaffins and long-chain polyamines that have the capacity to catalyze the precipitation of nanometer sized spheres of silica within minutes under physiologically relevant pH (Kröger et al. 1999, 2002). The silaffins, silacidins and long-chain polyamines form supramolecular assemblies with zwitterionic properties caused by the polyamine moieties and phosphate groups (Kröger et al. 1999, 2000, 2002; Wenzl et al. 2008; Tesson

and Hildebrand 2013). Silaffins are post-translationally modified polycationic phosphorylated peptides with a high proportion of hydroxyl amino acids such as serine and lysine residues that can form pentalysine clusters that can be linked by ε-amino groups to long-chain polyamines (Kröger et al. 2002; Poulsen et al. 2013). The long-chain polyamines (LCPAs) can have up to 20 repeated units and are the longest chains found in nature (Kröger et al. 2000). In *Cylindotheca fusiformis* the polyamines are attached to the polypeptide backbone of the silaffins, but in some other diatoms they may be linked to free amino acid derivatives (Kröger et al. 2000). Silacidins, are a class of acidic aspartate/glutamate and serine phosphate rich polypeptides formed from the post-translationally endoproteolytic processing of precursor polypeptides. Silacidins catalyze the precipitation of siliceous nanospheres in the presence of polyamines and silicic acid (Wenzl et al. 2008). Different combinations of silaffins and LCPAs under different combinations of pH and salinity have been shown to control the morphology of the nano-scale precipitated silica, forming blocks and spheres of different size, suggesting they may play a key role in guiding the formation of micro-scale structures associated with diatom frustule (Kröger et al. 2000). Hildebrand (2003) has hypothesized that the polypeptide backbone of silaffins may facilitate the formation of elongated structures while long chain polyamines may facilitate the formation of more complex molded structures. At present it is unclear whether the silica-precipitating peptides isolated from diatoms, such as the silaffins, silacidins and LCPAs, occur in other silicifying microalgal groups.

3.4 Transcriptomic Identification of Genes Associated with Silicification

Transcriptomic analyses of the differential expression of genes under Si-sufficient and low to limiting silicic acid conditions have identified a number of genes that may be involved in silicifiation in *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* (Mock et al. 2008; Sapriel et al. 2009; Shrestha et al. 2012). Mock et al. (2008) identified 159 up-regulated genes in *T. pseudonana* under low but not limiting silicic acid conditions. Seventy five of these genes were differentially expressed under the low silicic acid conditions but not under low iron, low nitrogen, low temperature or alkaline pH. Eighty four genes were differentially expressed under both low silicic acid and iron concentrations, suggesting there may be a molecular basis for elevated Si:C often observed in diatoms exposed to iron-limiting conditions. Many of the differentially expressed genes under low versus sufficient silicic acid conditions have no homology with known proteins and have trans-membrane spanning domains and/or secretory signals (Mock et al. 2008).

In a later study Shrestha et al. (2012) synchronized *T. pseudonona* cultures and analyzed differences in transcribed genes and expression levels under cell cycle arrest due to silicic acid limitation, during valve formation after silicic acid addition, and genes co-transcribed with silicic acid transporters *tpSIT1* and *tpSIT2*. Silicic acid starvation was associated with an over-representation of genes related to transcription and translation. The up-regulation of transcription and translation machinery in combination with high nitrogen stores maintained due to silicic acid stimulated cell cycle arrest may provide diatoms a competitive growth rate advantage over other phytoplankton groups once nutrient conditions improve (De La Rocha and Passow 2004; Shrestha et al. 2012). Frustule wall synthesis was associated with 485 differentially expressed genes, many putatively associated with cell signaling, protein degradation, extracellular proteins (including some with chitin binding domains), and genes whose products were potential precursors of silaffins, cingulins, SITs and choline and sugar transporters, and vesicle trafficking proteins that may be involved in the formation and function of the silica deposition vesicle. Twenty-four genes were differentially co-transcribed with *tpSIT1* and *tpSIT2*, many rich in repeated acidic amino sequences characteristic of silaffins and silacidins, and include a homolog of a silicon efflux transporter identified in higher plants (Shrestha et al. 2012).

The transcriptome of the fusiform morphotype of *P. tricornutum* was compared for cultures grown with (175 and 350 μM) and without silicic acid (Sapriel et al. 2009). The fusiform morphotype of *P. tricornutum* can incorporate Si fibers into the organic matrix of their cell wall but it does not strictly require silicic acid for growth and does not form a silicified frustule. The differential expression of 223 genes, 201 of which were up-regulated when silicic acid was present in the media were therefore assumed to be involved in the sensing, storage, and acquisition of silicic acid as opposed to frustule formation. Many of the putative genes that were significantly up-regulated by *P. tricornutum* in response to silicic acid were involved in pathways that can produce products known to be associated with silicification including: glycosylated trans-membrane proteins, silaffins and polyamines. Approximately a quarter of the over-expressed genes were expressed in clusters, including three of the four most highly over-expressed genes (16 to 330-fold) including two SITs, *ptSIT2-1* and *ptSIT2-2*. Sapriel et al. (2009) hypothesize that the ability to up-regulate genes in clusters may provide pennate diatoms with a selective advantage under temporally variable environmental conditions.

The transcriptomic analyses of Mock et al. (2008), Shrestha et al. (2012), and Sapriel et al. (2009) have identified many putative genes that may be important in silicification, many are newly discovered and need to be studied in more detail. Many of the genes identified show evidence of

translational and post-translational control, and vary considerably across the three studies, highlighting that the transcribed genome may be extremely sensitive to differences in experimental conditions.

4 Why Microalgae Silicify: The Costs and Benefits of Silicification

The construction and maintenance of complex siliceous structures, such as the diatom frustule, silicoflagellate skeleton, or scales covering chrysophytes and synurophytes requires a metabolic investment in silicic acid acquisition and establishment of the silicic acid gradient between the dilute external environment and the cell and SDV. Further costs are incurred in the construction and maintenance of silica transportation and deposition vesicles. In diatoms, and perhaps many other silicifying microalgae, there may be investment in silica-forming and silica-associated polypeptides and long-chain polyamines. A quantification of the costs associated with silicification is hampered by a fundamental lack of knowledge of the basic mechanisms responsible for silicification in most of the microalgal groups. In addition to direct metabolic and capital investment, the increased density of siliceous structures may increase sinking rate for phytoplankton. High sinking rates may reduce time in the upper sunlit water column and may reduce photosynthetic rate. Given these costs it seems logical there must be benefits associated with silicification (Knoll 2003).

Benefits attributed to silicified structures, specifically the diatom frustule, have been divided into three major categories (Finkel and Kotrc 2010): (I) siliceous structures may impede grazing, parasitoid or viral attack, (II) siliceous structures may positively promote the acquisition of growth-limiting resources or protect the cell from excess photon flux or UV or toxic metals, and (III) siliceous structures may alter sinking rate and this increased sinking rate may have a net positive effect on the availability of resources and interactions with predators, parasitoids and viruses. Specifically it has been argued that sinking may increase access to the nutrient-rich deep waters and shrink the nutrient-poor boundary layer around the cell, may facilitate sexual reproduction or resting stage formation, and may selectively remove cells infected by viruses or parasitoids (Smetacek 1985; Raven and Waite 2004). The balance of costs and benefits will be influenced by environmental and biotic conditions such as the availability of silicic acid concentrations in the environment and the degree of grazing pressure, that vary over space and time (Racki and Cordey 2000; Knoll 2003; Finkel et al. 2010; van Tol et al. 2012).

Biomineralized plankton have radiated over the last ~65 million years (Falkowski et al. 2004; Katz et al. 2004) and some silicified plankton (silicoflagellates, radiolarians, and perhaps the diatoms) have become less heavily silicified in response to declining surface silicic acid concentrations and increasingly armored (spines) in response to increased grazing pressure (Harper and Knoll 1975; Racki and Cordey 2000; Lazarus et al. 2009; Finkel et al. 2010; Finkel and Kotrc 2010; van Tol et al. 2012). It is commonly assumed that biomineralized skeletons provide protection against parasitoids and viruses and predators but quantitative measures are scarce (Hamm and Smetacek 2007). There is some evidence that microalgae covered by siliceous plates, such as diatoms or chrysophytes, synurophytes or the Parmales may be provided with some protection against digestion. External siliceous coverings, such as the diatom frustule or chrysophyte scale coverings, are not fully impervious to viruses or parasitoids, but may reduce the target area available for a viable attack and infection. The siliceous spines of diatoms and silicoflagellates and the spiny siliceous internal elements in Ebridia and dinoflagellates may have the potential to injure the digestive tract or feeding apparatus of grazers (Bell 1961; Thomsen and Moestrup 1985) and/or increase grazer handing time and the external skeleton may increase the effective size of some species removing them from the size range of some grazers. The phenotypic induction of increased silicification in diatoms when exposed to grazers is perhaps one of the strongest lines of evidence that diatom silicification may have evolved in response to grazing pressure (Pondaven et al. 2007). The origin of silicification may trace back to the Neoproterozoic vase-shaped microfossil *Melicerion poikilon*, possibly related to the Euglyphid amoeba (Porter et al. 2003; Porter 2011). These biomineralized eukaryotes appear as oxygen concentration and nitrogen availability and prey populations were on the rise, likely stimulating increased predation pressure and the evolution of defensive strategies (Knoll et al. 2007; Porter 2011).

The physical structure and chemical properties of the diatom frustule can influence resource acquisition and cell physiology. The silica frustule of diatoms alters both the scattering and absorption of light; silica frustules absorb UV and the micro-scale patterning of the frustule may alter the pattern of light within the cell (Kitchen and Zaneveld 1992; Davidson et al. 1994; Fuhrmann et al. 2004; De Stefano et al. 2007). The micro-scale structure of the diatom frustule has also been shown to influence the size sorting of particles at the frustule surface and nutrient diffusion rates (Hale and Mitchell 2001, 2002; Mitchell et al. 2013). In addition diatom silica may act as a pH buffer increasing the reaction rate of carbonic anhydrase (Milligan and Morel 2002) and frustulins in diatom silica may externally bind cadmium, suggesting silica may have the capacity to reduce the bioavailability of potentially toxic metals (Santos et al. 2013). The quantitative effect of these phenomena has not been clearly determined and it is unclear if these benefits apply to siliceous structures produced by groups of silicifying algae other than the diatoms.

5 Conclusions

Many taxonomically diverse organisms acquire Si and produce complex siliceous structures. Due to a lack of data it is unclear if there are significant differences in the genetic, molecular, and physiological mechanisms that allow the different taxonomic groups of microalgae to acquire, store and form siliceous structures. A comparative analysis of physiological responses to silicic acid concentrations and molecular and genetic mechanisms that control silicification across the Chromista would be expected to yield insight into the diversity of biological mechanisms that allow organisms to exploit Si and the evolutionary history of silicification.

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