Journal of
Nanoscience and Nanotechnology
Vol. 5, 1–9, 2005

RESEARCH ARTICLE

Approaches for Functional Characterization of Diatom Silicic Acid Transporters

Kimberlee Thamatrakoln and Mark Hildebrand*

University of California, San Diego, Scripps Institution of Oceanography, 9500 Gilman Drive MC 0208, La Jolla, California 92093-0208, USA

A major component of the diatom cell wall is silica, derived from silicon taken up from the environment. Due to limiting environmental concentrations of silicon, and a substantial requirement during cell wall synthesis, diatoms must transport silicon into the cell against a steep concentration gradient. This is accomplished through the silicic acid transporters (SITs). The SITs were first identified in the marine pennate diatom *Cylindrotheca fusiformis*. Five distinct SITs were found and have been classified as a novel family of transporters. This review covers our current understanding of silicon transport in diatoms with a focus on the SITs. Approaches for in-depth functional characterization of the SITs are discussed, including (1) isolating SITs from evolutionarily distant diatom species to identify conserved amino acids that may be important for function, (2) developing expression systems to assay the function of selected SITs, and (3) determining the cellular location and membrane topology of the *C. fusiformis* SITs to further clarify their roles in diatom silicon metabolism. Because of the specificity of interaction between the SITs and silicon, and the ability of the SITs to transport silicic acid across lipid bilayers, the SITs may have applications in nanotechnology.

Keywords: Diatom, Silicon, Silicic Acid Transporter (SIT), Silica, Germanium, Yeast Expression, Mutagenesis, Nanotechnology.

1. INTRODUCTION

Diatoms are unicellular, eukaryotic phytoplankton estimated to be responsible for up to 40% of marine primary productivity.[1] A limiting nutrient for diatom growth is silicon, a significant component of their cell walls.[2, 3] Diatoms are the major silicifying organisms on the planet, and along with silicoflagellates, radiolarians, and sponges convert 6.7 Gtons of soluble silicon into silica annually.[4, 5] Diatoms, long admired for the intricate microstructure of their cell wall, or frustule, are divided into two evolutionarily distinct morphological groups. As a general classification scheme, pennate diatoms are bilaterally symmetrical, whereas centric diatoms are radially symmetrical, although there can be significant deviations within each group. The reproducibility of a diatom species' silica morphology indicates the process of cell wall synthesis is genetically controlled.

The ability of diatoms to make complex nanoscaled three-dimensional silica structures offers attractive possibilities for their application in nanotechnology. In addition to the "final product," silica from the cell wall, it is important to consider other components involved in the cells' interaction with silicon. In particular, transport of silicon into the cell has been shown to be an integral part of frustule formation.[6, 7] Because of the specificity of transport, understanding its molecular details could be of benefit for nanotechnology. In this article we outline our current understanding and investigations into the mechanisms of diatom silicon transport. Information about the diatom species used in these studies is given in Table I.

2. SILICON TRANSPORT

The silica component of a diatom frustule can account for up to 50% of the dry weight of the cell depending on the species.[6, 8] As such, there is a high demand for silicon to be taken up from the environment. The predominant form of dissolved silicon in the ocean is orthosilicic

*Author to whom correspondence should be addressed.

Table I. Diatom species used in these studies.*

Abbreviation	Name	Type	No. of residues	Habitat
Cf SIT1	*Cylindrotheca fusiformis*	Pennate	320	Marine
Na	*Nitzschia alba*	Pennate	320	Marine
*N*spp	*N*. spp	Pennate	320	Marine
Np	*Navicula pelliculosa*	Pennate	320	Freshwater
Pt	*Phaeodactylum tricornutum*	Pennate	319	Marine
Tw	*Thalassiosira weissflogii*	Centric	324	Marine
Tp SIT1	*T. pseudonana*	Centric	324	Marine, freshwater
Tp SIT3	*T. pseudonana*	Centric	324	Marine, freshwater

* No. of residues indicates the number of residues used for comparative sequences analyses.

acid [$Si(OH)_4$]. Prior to the evolution of silicifying organisms, the world's oceans were nearly saturated with silicic acid (1.7–2.5 mM).[9, 10] Today, because of utilization by siliceous organisms, that number has dropped to a global average of 70 μM, but is generally <10 μM and sometimes <1 μM in surface waters.[4, 11] Diatoms are thus faced with the challenge of obtaining limiting amounts of silicic acid; with intracellular concentrations ranging from 19 to 340 mM,[11] they must transport it into the cell against a steep concentration gradient. Furthermore, diatoms maintain supersaturated intracellular levels of silicic acid in an unpolymerized, soluble form. It has been proposed that this occurs by association of intracellular silicon with organic silicon binding components or proteins.[6, 11]

Numerous studies on silicic acid uptake in diatoms show that the rate follows Michaelis-Menten kinetics with K_s values between 0.2 and 7.7 μM and V_{max} ranging from 1.2 to 950 fmol of Si $cell^{-1}h^{-1}$.[11–17]

The predominant transported form of silicon is orthosilicic acid, but there is evidence in one species, *Phaeodactylum tricornutum*, that the anionic form $SiO(OH)_3^-$ can also be transported.[18, 19] Silicic acid is cotransported with sodium in marine diatoms and some data suggest it may be sodium and potassium coupled in freshwater species.[15, 17] However, in freshwater, higher K_s values and increased silicon requirements[20] bring into question the extent of ionic coupling.[21] Regardless, transport under marine conditions has the characteristics of a sodium/silicic acid symporter. Transport appears to be electrogenic with a 1:1 ratio of $Si(OH)_4$:Na^+.[17] Studies have shown that exposure to sulfhydryl blocking reagents inhibits transport.[13, 15]

Three modes of silicon uptake have been proposed based on chemostat studies; surge uptake, externally controlled uptake, and internally controlled uptake.[22, 23] Surge uptake occurs when silicate-starved cells are replenished with silicon. Under these conditions, intracellular silicon pools are depleted and the silicon concentration gradient into the cell is at its maximum. Measurements during this mode show maximum uptake rates. Externally controlled uptake occurs when extracellular levels of silicon are low and the rate of uptake is controlled by the substrate concentration. In internally controlled uptake, the intracellular utilization of silicon, inferred to be the rate of silica deposition into the cell wall, controls the rate of uptake.[7, 22, 23] Thus, there is a temporal coupling between uptake and incorporation. This has been seen in most diatoms, but there are exceptions.[24]

The coupling of uptake and incorporation was thought to be regulated by intracellular pools of silicon.[22, 23] It was proposed that if silicate were added to limited cells, surge uptake would occur, increasing the levels of intracellular silicon to a maximum. This would induce a negative feedback mechanism by which transport would decrease. However, in *Cylindrotheca fusiformis*, control of transport occurs before intracellular silicon reaches maximum levels,[6, 7] suggesting that other cellular factors regulate the rate of transport. A new hypothesis proposes that uptake is controlled by the ratio of bound:unbound silicon inside the cell.[6] When intracellular silicon binding components are in excess, uptake occurs, and when levels of unbound silicon are higher than those of bound silicon, uptake is inhibited or efflux induced.

Efflux is an often overlooked and under-appreciated aspect of diatom silicon metabolism.[15, 25, 26] An excess of unbound silicon could be detrimental to a cell because, at intracellular pH, silicon polymerizes at concentrations above 2 mM.[9] Thus, diatoms must have a means to remove excess unbound silicon. Data show that efflux only occurs in the presence of external silicon and increases with increasing external silicon.[15] As silicon concentrations outside the cell rise, increasing surge uptake would occur, increasing the levels of unbound intracellular silicon and inducing efflux to a greater extent.

After silicic acid enters the cell, the mechanism of intracellular transport is relatively unknown. Silicic acid must make its way from the plasma membrane through the cytoplasm to the silica deposition vesicle (SDV). It must then cross the silicalemma into the SDV where it enters an acidic environment.[27] Through mechanisms currently being elucidated, silicic acid is polymerized to form the silica of the new cell wall.[28–31]

Measurements of uptake kinetics in whole diatom cells and extracts have provided critical information about silicon transport, but understanding details of the mechanism of transport requires a molecular approach. This requires identifying the genes, and their respective

proteins, involved in silicon recognition and transport. The first step toward developing a molecular model for silicon transport was the cloning and characterization of the silicic acid transporters.[32, 33]

3. IDENTIFICATION OF THE SILICIC ACID TRANSPORTERS

Genes encoding silicic acid transporters (SITs) were first identified in the marine pennate diatom *C. fusiformis.*[32] Originally named *silicon* transporters, it was later discovered that silicic acid was the predominant form of transported silicon[19] and they are thus more accurately referred to as *silicic acid* transporters. The function of a SIT cDNA was assayed by microinjecting SIT mRNA into *Xenopus laevis* oocytes and measuring uptake.[32] Uptake was sodium-dependent, specific for silicon and germanium (a commonly used radiotracer for silicon),[25, 34] and sensitive to sulfhydryl blocking reagents, consistent with diatom whole-cell uptake experiments. The SITs were among the first silicon-responsive genes to be identified.[35] Five SIT genes (SIT1–5) were found in *C. fusiformis*, each with a distinct level or pattern of expression during cell wall synthesis.[33] They represent a novel class of transporter and are the first proteins shown to specifically recognize silicic acid.[32] Analysis of their sequence reveals the presence of a sodium symporter signature sequence, consistent with previous findings that silicon uptake is coupled to Na^+.[6, 17] This sequence has been found in a number of other Na^+ symporters and was thought to play a role in cation binding.[36] However, later studies found this sequence is dispensable with regards to cation binding, and other residues, not conserved between classes of transporters, have been identified as playing key roles in Na^+binding.[37–40]

The *C. fusiformis* SITs can be divided into two major domains, an N-terminal region containing ten transmembrane segments and their connecting intra- and extracellular loops and a C-terminal coiled-coil region. As in other even-numbered transmembrane domain transporters, both the N- and C-terminus are predicted to be located intracellularly.[41, 42] Within the transmembrane region, the SITs are highly conserved, having 87–99% amino acid identity. Passage of silicic acid must occur in the transmembrane region. The sensitivity of transport to sulfhydryl blocking reagents[15, 17] suggests a role for cysteine residues, and there are ten cysteines within the membrane-spanning region, nine of which are conserved among all five SITs. Cysteines are known to play a stabilizing role in protein structure by their ability to form disulfide bonds and they can be involved in binding metals. Although silicon uptake has been shown to be affected by zinc and copper,[43, 44] no transporters have yet been identified that utilize a metal cofactor. Other conserved residues within the transmembrane region may play a role in recognition, binding, and transport of silicic acid. However, the high level of conservation within this region in the *C. fusiformis* SITs precludes identification of such residues. The C-terminus is less conserved with 39–67% amino acid identity and is predicted to form a coiled-coil motif, a structure know to play a role in protein–protein interactions. In other transporters, the C-terminus has been shown to play a role in regulating transporter activity, determining intracellular localization, or controlling conformational changes during transport.[45–49] The sequence disparity within this region among the five SITs could be responsible for functional differences.

While the specific role of each SIT has yet to be determined, measurements of mRNA have shown varying levels of gene expression during the period of cell wall synthesis.[33] In synchronized cultures of *C. fusiformis*, all SITs are maximally expressed prior to silicon incorporation into the cell wall and follow the pattern: SIT4>SIT2>SIT1>SIT5>SIT3, where SIT4 is the most highly expressed and SIT3 the least. While the overall level of expression differs among the five SITs, the pattern of expression is strikingly similar for SIT2-5, suggesting coordinate regulation of these genes. The pattern of SIT1 expression differs in two ways. First, mRNA levels do not peak to the same degree as the other SITs. Second, levels remain high well after levels of SIT2-5 have dropped.

The identification of the silicic acid transporters was an important step in elucidating diatom silicon metabolism. It is suggested the five SITs have differing affinities or capacities for transport and are proposed to play specific roles in silicic acid transport.[33] However, these hypotheses can only be tested by more in-depth studies on their function.

4. COMPARISON OF SIT SEQUENCES

The SITs specifically interact with silicon, but how they recognize, bind, and transport silicic acid is still unknown. Amino acid residues important for the function of a protein are likely conserved during evolution. A survey of SIT sequences from evolutionarily distant diatoms and possibly other silicifying organisms could reveal conserved amino acids directly involved in transport.

To isolate SIT sequences from other diatoms, primers were designed based on conserved residues with low degeneracy in the *C. fusiformis* SITs. These primers amplified 1 kbp (kilobase pair) PCR products from both pennate and centric diatoms, from marine and freshwater environments. These products were sequenced, confirmed to be SITs based on BLAST homology search,[50] and analyzed using ClustalW, a program for performing multiple sequence alignments.[51] Results of pairwise comparisons are shown in Table II. Comparison between individual SITs indicated 50–85% amino acid identity. Comparison of all pennate SIT sequences showed 42% identity, whereas centric SIT sequences were 53% identical. However, when all pennates and centrics were compared, only 28% were conserved. In addition to conservation of single

Table II. Pairwise comparison of diatom partial SIT amino acid sequence.*

	% identical (% similar)						
	Na	*Np*	*N*spp	*Pt*	*Tw*	*Tp* SIT1	*Tp* SIT3
cf SITI	79 (94)	76 (94)	59 (87)	62 (89)	56 (87)	55 (86)	54 (85)
Na		77 (94)	57 (86)	59 (86)	56 (84)	52 (82)	53 (83)
Np			56 (84)	59 (85)	57 (85)	54 (84)	55 (83)
*N*spp				69 (22)	58 (87)	55 (84)	55 (83)
Pt					60 (87)	57 (87)	55 (84)
Tw						85 (96)	60 (86)
Tp SITI							57 (85)

* Percent identical refers to conserved amino acids. Percent similar includes conserved amino acids in addition to conserved substitutions. Numbers generated using ClustalW protein alignment tool. See Table I for abbreviations. Because the five *Cf* SITs are almost 90% identical in this region, only SIT1 is shown for comparison. Genomic sequencing of the centric diatom *Tp* revealed the presence of three SITs, denoted SIT1–3. *Tp* SIT2 is not shown due to high conservation with SIT1.

amino acids, areas containing up to seven consecutively conserved amino acids were found. These are candidate regions for playing a direct role in silicon uptake and will be of high priority for future studies.

An unrooted maximum parsimony phylogenetic tree, constructed based on multiple sequence alignment of the 1 kbp SIT sequence (Fig. 1), showed that all SITs from pennate species grouped together, as did the centric species. The three SIT sequences for *Thalassiosira pseudonana* were obtained from the genome sequencing project (http://genome.jgi-psf.org/thaps0/thaps0.home.html). An interesting feature was the divergence of *T. pseudonana* SIT3 from the other two SITs, in contrast to the high similarity among the five *C. fusiformis* SITs. The placement of *Phaeodactylum tricornutum* and *Nitzschia* spp. on the same branch, but separate from the other pennate SITs, is also of interest. Although the *Nitzschia* species used was unidentified, it did not group with its sister species, *Nitzschia alba*. It is interesting to speculate whether the placement of *P. tricornutum* away from the other pennates is related to its ability to transport the less abundant anion of silicon, $SiO(OH)_3^-$, or is because this species does not have an obligate requirement for silicon.[19]

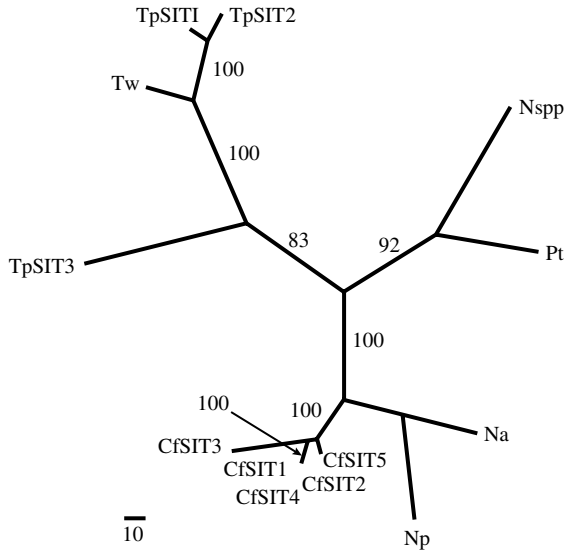

Fig. 1. Unrooted maximum parsimony phylogenetic tree based on an amino acid alignment of the 1 kbp SIT PCR product. See Table I for abbreviations. Bootstrap values are shown at internal branches (100 replicates, values >80% shown). Scale bar = 10 substitutions.

While obtaining a partial sequence of the SITs is providing useful information, it will be important to obtain the full-length sequence of representative SITs. The 1 kbp fragment only contains transmembrane segments 3–10 and lacks the C-terminus, which is highly variable in the five *C. fusiformis* SITs. The three SITs identified by genome sequencing of the centric diatom *T. pseudonana* apparently lack the predicted C-terminal coiled-coil region of the *C. fusiformis* SITs, indicating a possible alternative regulatory mechanism. Full-length SIT sequences are currently being obtained through the construction and screening of genomic libraries.

5. CHARACTERIZING THE FUNCTION OF INDIVIDUAL SITS

Sequence analysis and structure prediction of the SITs must ultimately be related to function. It would be ideal to assay the function of each SIT individually in its native species. However, the presence of multiple SITs complicates data interpretation and makes the assignment of function to a particular SIT difficult. In addition, techniques for genetically manipulating diatoms, to express one SIT at a time, for example, are currently limited.[21] Existing methods to characterize transporter proteins outside their native host include expression in a heterologous host and reconstitution in lipid vesicles.

A key feature in selecting a heterologous system is that the organism should be eukaryotic. Because the SITs are predicted to be membrane-associated proteins, correct targeting may be crucial for their function. Although other diatom proteins possibly involved in transport may not be

present in a heterologous host, past experiments in *Xenopus* oocytes indicate the transporters are able to function without such proteins.[33]

We are exploring the yeast, *Saccharomyces cerevisiae*, as a system to characterize the SITs. *S. cerevisiae* provides numerous advantages over other heterologous hosts in that it is easy to grow in large quantities, is amenable to genetic manipulation, and is a well-developed expression system so a number of kits for transformation and protein expression are commercially available. A possible problem with *S. cerevisiae* is its endogenous ability to transport silicic acid (Hildebrand, unpublished), which likely occurs nonspecifically through another transporter but could interfere with measurements of SIT activity. Although studies on other transporters have been done without removing endogenous uptake,[52] measurements would be more accurate if this uptake could be drastically reduced or eliminated. A similar approach has been used with other transporters, such as the *Chlorella* HUP1 hexose transporter,[53–56] *Escherichia coli* Na^+/proline symporter,[37] and wheat HKT1 K^+/Na^+ symporter.[57]

Germanium, in the form of germanic acid, has been used in past studies as a tracer for silicic acid[15, 16, 25, 34] and is also known to be toxic to yeast and bacteria.[58] This provides us with a tool with which to generate and identify mutants with impaired endogenous silicic acid uptake. Yeast cells were mutagenized with ethyl methyl sulfonate and screened for their ability to grow in the presence of germanium concentrations toxic to wild-type cells. Four mutants were obtained that grew under concentrations of germanium 6-fold higher than in wild-type. While these mutants may be deficient in their ability to uptake germanium, it is possible their mutation is located at a downstream effector. To test this, uptake measurements will be done using radiolabeled germanium (^{68}Ge), where cells deficient in uptake will not accumulate ^{68}Ge. We will subsequently transform mutants deficient for uptake to express each SIT individually. If they are functional, the mutants will regain sensitivity to germanium and will provide us with a system with which native and modified SITs can be assayed.

Another possible technique for studying individual SIT function is reconstitution of purified SIT protein in artificial lipid membranes, or liposomes. Spherical liposomes provide a cell-free system where both internal and external conditions can be tightly controlled. This approach has been used for studying membrane transporters such as the *Chlorella* HUP hexose/H^+ transporter,[47] *E. coli* SstT Na^+/Serine symporter,[59] and the *Arabidopsis thaliana* STP monosaccharide/H^+ symporter.[60] We propose to express the SITs in yeast as 6-His tag fusion proteins, which will allow purification of the protein over a nickel affinity column. Various reconstitution techniques are available.[61–63] and methods for unidirectionally reconstituting transporter proteins have been established.[64, 65] Being able to control the orientation of transporter insertion into the lipid vesicle would provide us with a tool to study both uptake and efflux.

Measurements of uptake and efflux rates are possible through the use of radioactive tracers, such as germanium (^{68}Ge) and silicon (^{32}Si).[25, 34, 66] For example, yeast transformed to express a SIT can be incubated in the presence of radioactive germanium. After a period of time, cells would be harvested and uptake of labeled material would be measured using a gamma counter. Uptake of radiolabeled germanium can then be equated to silicic acid uptake.[15, 16] Efflux can be measured by preloading cells with the radiotracer and measuring radioactivity retained by the cells. Loss of intracellular radioactivity can be translated into rates of efflux.[15] Variations on these basic measurements will allow us to define detailed parameters of transport of the native and modified SITs.

The functional activity of the SITs (or any protein) is determined by their amino acid sequence. To directly test the importance of conserved residues in the SITs, site-directed mutagenesis can be applied. This PCR-based technique allows one to directly modify specific amino acid residues to determine the effect of these changes on function.[67, 68] By modifying single amino acids or groups of amino acids, we can determine the effect on transport using the systems described above.

6. DETERMINING SIT TOPOLOGY AND INTRACELLULAR LOCALIZATION

The predicted transmembrane topology of the SITs is based on computer-generated hydropathy plots. It is also inferred from comparisons with other even-numbered transmembrane domain transporters. While the SITs are clearly membrane-associated proteins, it is unknown whether they all target to the plasma membrane or if some remain associated with intracellular membranes such as the silicalemma. These issues of topology and intracellular localization can be answered by employing the use of antibodies and fusion tag proteins.

Antibodies were generated by injecting rabbits with a conjugated peptide derived from the conserved region between transmembrane segment 7 and 8 of the *C. fusiformis* SITs. As the fourth extracellular loop, it is denoted EL4.[6] EL4 was chosen because it is the largest of the hydrophilic loops and its predicted extracellular localization would allow it to be accessible in whole cell labeling experiments. Predictive programs also suggest this region is highly antigenic, increasing the likelihood of inducing an immune response and producing antibodies. One aspect of EL4 antibodies is that this region is conserved among the five SITs and thus will not be able to distinguish one from the other. Nonetheless, this antibody can provide general information on localization. We are currently testing the specificity of the EL4 antibodies for

the SITs, and if successful, we can begin to characterize SIT localization.

The predictive model for SIT topology can be tested in whole cell labeling experiments using fluorescently labeled secondary antibodies and microscopy to determine whether the region recognized by the EL4 antibody is located on the extracellular surface. Alternatively, membrane vesicle preparations can be probed with the antibody to determine the orientation of the transporter. Diatom membrane vesicles, prepared by osmotic lysis and centrifugation, were used to determine the role of various ion gradients in silicon uptake.[17] Sidedness is determined by monitoring Na^+/K^+ ATPase activity in the presence and absence of detergents. Similar techniques were used to determine the topology of the *E. coli* membrane-bound lactose carrier protein[41] and PutP Na^+ proline symporter[69] and the human erythrocyte glucose transporter.[42]

While the SITs are clearly membrane-associated proteins, it is unclear if any of them are intracellularly localized. One method for determining this is to generate fluorescently tagged SITs. Fusions between the SITs and fluorescent proteins, such as green fluorescent protein (GFP), can be made, which, after introduction into the cell, can be monitored by fluorescent microscopy. Tagging with GFP has been used to study other transporters, such as the glucose transporter GLUT4.[70] A SIT-GFP fusion can be generated and used to transform diatoms by using established techniques.[70–72] Confocal microscopy can follow GFP fluorescence to determine the localization of the SITs. This approach was successfully applied in the diatom *Phaeodactylum tricornutum* transformed to express a nonnative transporter.[72] In addition to GFP, we can take advantage of the numerous other fluorescent proteins now commercially available.[73] For example, fluorescent proteins such as RFP (red), YFP (yellow), and CFP (cyan) can be used to tag different SITs and expression of each can be monitored simultaneously.

Determining SIT topology and localization could have important implications for understanding SIT function. For example, as the SITs are released from the Golgi complex and transported in Golgi vesicles, they must be maintained in their proper orientation (Fig. 2). A SIT targeted to the plasma membrane will embed itself with the predicted hydrophilic C-terminus in the cytoplasm. Thus uptake would occur across the plasma membrane toward the C-terminus. A SIT targeted to the silicalemma would likewise get embedded with the C-terminus in the cytoplasm, but transport into the SDV would have to occur in the direction facing away from the C-terminus. While the SITs are capable of transport in both directions, it is unlikely that transport in both directions is sodium-coupled. Intracellular sodium concentrations are too low to allow sodium symport into the SDV, as occurs for uptake across the plasma membrane. Thus, intracellularly located SITs would use a different driving force for transport.

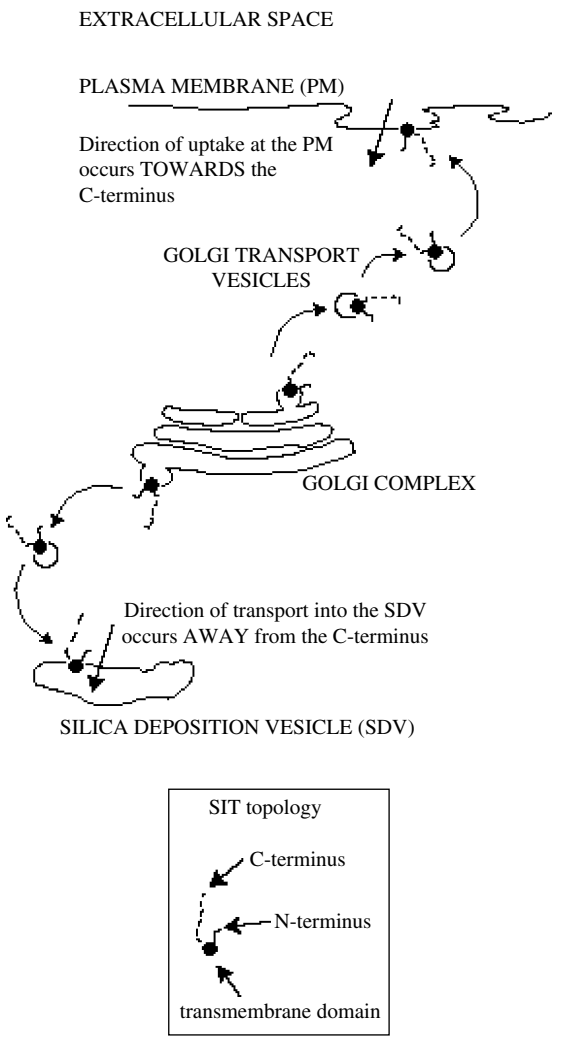

Fig. 2. Proposed route and orientation of SIT proteins from the Golgi complex to the plasma membrane (PM) and the silica deposition vesicle (SDV).

7. POSSIBLE APPLICATIONS OF SIT RESEARCH IN NANOTECHNOLOGY

There are two possible applications in which research on the SITs could benefit nanotechnology. The first is the use of the SITs as silicic acid delivery tools across membranes. The second is to utilize information gained from identification of silicic acid binding amino acids for control and catalysis of reactions involving silicon.

As described above, one system for characterizing the SITs involves reconstitution of expressed SITs in artificial lipid membranes. There is substantial literature on the use of surfactant-based liquid crystal materials for formation of mesoporous silica.[74–76] Incorporation of the SITs into lipid bilayer membranes could enable the specific transport of silicic acid into micellar structures or across planar membranes. By understanding the detailed mechanism of transport, one could adjust conditions to specifically deliver desired amounts of silicic acid across a bilayer

membrane. Because substrate availability is a major controlling factor in chemical reactions, this could be very useful.

As mentioned, the SITs are the first proteins shown to directly and specifically interact with silicic acid.[32] As such, they are a potential model system for understanding how proteins in general may recognize and bind silicon. This information could be useful in biomimetic approaches to materials synthesis, both in terms of the coordination and binding of silicon, and also in possible mechanisms of enzyme catalysis involving silicon. A hallmark of biology is the specific interaction between proteins and their substrates. In the case of enzymes, such interactions stabilize transition states and lower activation energies, resulting in catalysis under ambient biological conditions. The specificity of these interactions is a result of the three-dimensional arrangement of amino acids in a protein relative to the interacting substrate. Thus, identifying candidate silicon-interacting amino acids in the SITs could be useful. Although challenging to accomplish, the three-dimensional structures of several transport proteins have been resolved.[77–80] Determining the three-dimensional structure of a SIT would greatly clarify the transport mechanism and result in a detailed understanding of the coordination between silicic acid and interacting amino acids. Such information could be invaluable in devising schemes to lower reaction energies in in vitro syntheses.

Acknowledgments: We thank Grace Lim for assistance with phylogenetic tree construction and Michael Latz for reading the manuscript. This work is supported by a subproject of the Center for Environmental Bioinorganic Chemistry from Princeton University and an Art Proceeds Fellowship.

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