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REVIEW

Role of urea in microbial metabolism in aquatic systems: a biochemical and molecular review

Caroline M. Solomon[1,*], Jackie L. Collier[2], Gry Mine Berg[3], Patricia M. Glibert[4]

[1]Department of Biology, Gallaudet University, 800 Florida Avenue NE, Washington, DC 20002, USA
[2]School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794-5000, USA
[3]Department of Environmental Earth System Science, Stanford University, 397 Panama Mall, Stanford, California 94305, USA
[4]University of Maryland Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, Maryland 21613, USA

ABSTRACT: Urea synthesized commercially and formed naturally as a by-product of cellular metabolism is an important source of nitrogen (N) for primary producers in aquatic ecosystems. Although urea is usually present at ambient concentrations below 1 µM-N, it can contribute 50% or more of the total N used by planktonic communities. Urea may be produced intracellularly via purine catabolism and/or the urea cycle. In many bacteria and eukaryotes, urea in the cell can be broken down by urease into NH_4^+ and CO_2. In addition, some bacteria and eukaryotes use urea amidolyase (UALase) to decompose urea. The regulation of urea uptake appears to differ from the regulation of urease activity, and newly available genomic sequence data reveal that urea transporters in eukaryotic phytoplankton are distinct from those present in *Cyanobacteria* and heterotrophic bacteria with different energy sources and possibly different enzyme kinetics. The diverse metabolic pathways of urea transport, production, and decomposition may contribute to differences in the role that urea plays in the physiology and ecology of different species, and in the role that each species plays in the biogeochemistry of urea. This review summarizes what is known about urea sources and availability, use of urea as an organic N growth source, rates of urea uptake, enzymes involved in urea metabolism (i.e. urea transporters, urease, UALase), and the biochemical and molecular regulation of urea transport and metabolic enzymes, with an emphasis on the potential for genomic sequence data to continue to provide important new insights.

KEY WORDS: Urea · Urea transport · Urease · Urea amidolyase · Urea cycle · Purine catabolism

INTRODUCTION

The importance of urea, and other regenerated forms of nitrogen (N), in phytoplankton production and its inclusion in estimates of regenerated production has long been recognized (sensu Dugdale & Goering 1967); however, there were few available data on urea uptake by phytoplankton until the 1970s (McCarthy 1972). Although urea is usually present at ambient concentrations below 1 µM-N in aquatic ecosystems, it is an important source of regenerated N and a major substrate for phytoplankton nutrition (e.g. Antia et al. 1991, Berman & Bronk 2003). The natural role of urea in coastal ecosystems is now being strongly affected by anthropogenic enrichment of urea, which appears to be associated with increasing occurrences of some harmful algal blooms (HABs; Glibert et al. 2006). Thus, gaining a better understanding of urea sources, sinks, and dynamics is an important goal in aquatic microbial ecology. This review supports that effort by integrating the current state of knowledge on biochemical processes and regulation with newer information from recent genomic sequencing projects. Specifically, this review covers urea sources and availability, use of urea

*Email: caroline.solomon@gallaudet.edu

as an organic N growth source, rates of urea uptake and enzymatic activity in urea metabolism (i.e. urease, UALase), and the biochemical and molecular regulation of urea transport. Much research is needed to more fully understand the links between the biochemistry of urea and its importance in the ecology of aquatic systems.

UREA SOURCES, AVAILABILITY, AND RATES OF UPTAKE AND GROWTH

Sources of urea to aquatic ecosystems

Urea in aquatic ecosystems comes from both natural and anthropogenic sources. Among the natural sources of urea in the water column are regeneration by heterotrophic bacteria (Mitamura & Saijo 1981, Cho & Azam 1995, Cho et al. 1996, Berg & Jørgensen 2006), excretion by macro- and microzooplankton (Corner & Newell 1967, Mayzaud 1973, Bidigare 1983, Miller & Glibert 1998, L'Helguen et al. 2005, Miller & Roman 2008, Painter et al. 2008), and release by phytoplankton (Hansell & Goering 1989, Bronk et al. 1998, Bronk 2002). Urea is also produced by benthic heterotrophic bacteria and macrofauna and released from sediments into the water column (Lomstein et al. 1989, Lund & Blackburn 1989, Pedersen et al. 1993, Therkildsen et al. 1997). The supply of regenerated urea may vary daily as copepods such as *Acartia tonsa* excrete more urea during night-time hours (Miller & Glibert 1998, Miller & Roman 2008). Urea regeneration rates under some conditions may exceed rates of NH_4^+ regeneration (e.g. Chesapeake Bay; Bronk et al. 1998). It has also been shown that macrofauna such as sharks (McCarthy & Kamykowski 1972), aggregations of larval fish (McCarthy & Whitledge 1972), and seabird guano can be sources of urea on local scales (Harrison et al. 1985). Urea also enters aquatic ecosystems via atmospheric deposition (Timperley et al. 1985, Cornell et al. 1995, Peierls & Paerl 1997, Mace et al. 2003) and in this form may come from both natural and anthropogenic sources.

In addition to the many natural sources, anthropogenic urea that enters aquatic ecosystems is produced commercially via the Wöhler process in which silver cyanate and ammonia are combined (Smil 2001). Serendipitously discovered in 1828, this chemical synthesis of urea has led to industrial production that now nears 70 million metric tons yr^{-1} (Berman & Bronk 2003, Glibert et al. 2006). Worldwide use of urea has increased more than 100-fold in the past 4 decades (Glibert et al. 2006). Of its commercial use, the dominant application is as a fertilizer. Urea is also used as a feed additive, in herbicides and pesticides, as a deicer for airplanes, and in bioremediation (Glibert et al. 2006).

Urea availability in aquatic ecosystems

Urea concentrations in aquatic ecosystems are variable, ranging from nondetectable (less than ~0.05 µM-N) to tens of µM-N measured using standard colorimetric techniques (e.g. McCarthy 1970, Holmes et al. 1999, Revilla et al. 2005). The fact that each molecule of urea contains 2 N atoms has contributed to some confusion in the literature with respect to units and concentrations. Some authors refer to concentrations of 'urea' while others refer to concentrations of 'N', and in many cases the authors do not clearly specify which unit was used. The resulting 2-fold uncertainty can sometimes make comparisons difficult. Units of 'N' are used herein.

Oceanic concentrations of urea are typically very low, and often nanomolar-N concentrations are measured (Bronk 2002, Painter et al. 2008). In contrast, concentrations of urea in coastal, estuarine, and riverine systems are quite variable, and concentrations as high as 25 to 50 µM-N have been reported in tributaries of the Chesapeake Bay (Lomas et al. 2002, Glibert et al. 2005), nearshore waters adjacent to the heavily fertilized Yaqui Valley, Mexico (Glibert et al. 2006), Santa Cruz, California (Kudela et al. 2008), and the Knysna Estuary, South Africa (Switzer 2008). Freshwater concentrations of urea are similar to those in coastal ecosystems and are quite variable. Urea concentrations vary from undetectable to 6–11 µM-N in Lake Kinneret, Israel (Berman 1974), 0.24–1.17 µM-N in Lake Biwa (Mitamura & Saijo 1981), and 0.02–50 µM-N in Polish lakes (Siuda & Chróst 2006). Urea concentrations are higher in mesotrophic than eutrophic Polish lakes (Siuda & Chróst 2006). In general, urea concentrations in aquatic ecosystems are less than those of NO_3^- and NH_4^+, but may exceed the concentrations of these inorganic N forms on occasion, and for short periods of time, particularly when runoff occurs from heavily fertilized areas (Glibert et al. 2001, 2006, Kudela et al. 2008, Switzer 2008).

Rates of urea uptake

Despite the fact that urea is often present in lower concentrations than NO_3^- and NH_4^+, in coastal and oceanic ecosystems, rates of urea uptake can be greater than rates of inorganic N uptake and can account for more than 50% of N uptake by planktonic communities (McCarthy 1972, Kaufman et al. 1983, Glibert et al. 1991, Berg et al. 1997, Bronk et al. 1998, Kudela & Cochlan 2000, Berman & Bronk 2003). Urea uptake rates in aquatic systems range from <0.1 nM-N h^{-1} to 3.6 µM-N h^{-1} (Kristiansen 1983, Berg et al. 1997, Lomas et al. 2002, Berman & Bronk 2003). Sometimes,

rates of urea uptake can meet most of the phytoplankton demand for N. For example, urea has been observed to support a large fraction of the N demand of many HABs including the dinoflagellate *Lingulodinium polyedrum* off the coast of Mexico (Kudela & Cochlan 2000), the dinoflagellate *Alexandrium catenella* in Thau Lagoon in Southern France (Collos et al. 2004), and the pelagophyte *Aureococcus anophagefferens* in Great South Bay and Peconic Bay, New York (Lomas et al. 1996, Berg et al. 1997, Gobler et al. 2002).

Heterotrophic bacteria can be both producers and consumers of urea, and their contribution to urea uptake relative to that of phytoplankton is often difficult to assess (Jørgensen 2006). Uptake of urea can account for a small (<3%) to large (41%) part of total bacterial N uptake (Wheeler & Kirchman 1986, Kirchman et al. 1991, Jørgensen et al. 1999a, Jørgensen 2006). The percentage contribution of bacteria to total plankton community urea uptake also varies from small (<10%; Cho & Azam 1995, Cho et al. 1996) to large (>80%; Middelburg & Nieuwenhuize 2000). The variability in contribution by bacteria to total microbial urea uptake may reflect variability in their roles in an ecosystem. For example, in a transect in the Gulf of Riga, bacteria produced 53% of the urea near the shore and progressively became consumers offshore where they utilized 20% of the urea (Jørgensen et al. 1999b).

Phytoplankton rates of growth on urea as sole N source

Some field observations have suggested that urea may promote the growth of particular phytoplankton over others (e.g. flagellate species over diatoms; Antia et al. 1991, Glibert & Terlizzi 1999, Berg et al. 2003). Many algal species have been found to grow well on urea, and for most species surveyed there appears to be little difference in growth rate for cells grown under comparable environmental conditions using NO_3^-, NH_4^+, or urea as the sole growth source of N (Table 1), although Berman & Chava (1999) reported much higher rates on urea than NO_3^- and NH_4^+ for 3 *Cyanobacteria*, a diatom, and a chlorophyte. For some species, such as *Emiliania huxleyi* (Pustizzi et al. 2004, Strom & Bright 2009) and *Aureococcus anophagefferens* (Berg et al. 2008), there appear to be differences among clones in their rate of growth on urea. In some cases, growth was faster on urea than NO_3^- and NH_4^+ at a lower irradiance; this pattern could be seen for *Chaetoceros gracilis, Dunaliella tertiolecta,* and *Heterosigma akashiwo* (Levasseur et al. 1993, Herndon & Cochlan 2007; Table 1). The wide range of growth conditions used by different investigators prevents further comparisons of growth of different species on urea.

The phytoplankton included in Table 1 can all grow well on urea, but some species cannot. Previous investigators have noted that some diatoms cannot use urea as a source of N for growth (Neilson & Larsson 1980). More recently, Hildebrand & Dahlin (2000) reported complete growth arrest of the diatom *Cylindrotheca fusiformis* when its sole N source was switched from NO_3^- to urea and its NO_3^- transporter was concomitantly up-regulated to the same level as in N-starved cultures. Some phytoplankton may not grow on urea, because they do not possess enzymes essential for urea uptake and/or catabolism. The genes encoding the enzyme urease were shown to be essential for the marine cyanobacterium *Synechococcus* strain WH7805 to grow on urea, while a *Synechococcus* strain that could not grow on urea (WH7803) lacked the enzyme urease (Collier et al. 1999). Some strains of *Prochlorococcus* do not contain the urease gene in their genomes (e.g. strains MIT9211, MIT8515, CCMP1375), but no physiological data are available to determine if these strains cannot grow on urea. There is apparently a great deal of variation in the use of urea relative to other N sources, with some phytoplankton growing best on urea, others growing better on other N sources, and still others unable to utilize urea at all. Additional work is needed to better describe urea utilization by more different phytoplankton taxa, and to understand the molecular genetic bases for differences among them.

A comparable body of literature on the growth of heterotrophic bacterial species is needed to understand their importance in aquatic ecosystems when using urea and other forms of N. Research in this area would complement our understanding of phytoplankton N nutrition and assist in the interpretation of field data.

UREA METABOLISM IN BACTERIA AND PHYTOPLANKTON

Molecular mechanisms of urea transport

Because ambient concentrations are generally low, urea mostly enters a microbial cell from external sources by active transport (Fig. 1). Urea active transporters have been identified in a number of bacteria and phytoplankton. In several prokaryotes, including the actinobacterium *Corynebacterium glutamicum* and *Cyanobacteria*, urea uptake occurs via ABC-type (ATP-binding cassette) transporters that use energy from ATP to transport urea across the cell membrane and are encoded by the *urtABCDE* operon (Beckers et al. 2004, Su et al. 2005; Table 2). Energy-dependent urea transporters have been described in several other heterotrophic bacterial species, including *Deleya* (now *Halomonas*) *venusta* HG1, *Pseudomonas aeruginosa,*

Table 1. Estimates of growth rate (μ) from studies in which a unialgal culture was grown using NO_3^-, NH_4^+, or urea as the sole N substrate. Where standard deviations (SD) are not given, they either were not reported in the original report or were difficult to accurately extract from the reported graph

Species	Light level (μmol photons m^{-2} s^{-1})	NO_3^- μ (d^{-1})	±SD	NH_4^+ μ (d^{-1})	±SD	Urea μ (d^{-1})	±SD	Source
PHOTOSYNTHETIC BACTERIA								
Cyanophytes								
Synechococcus sp.[a]	80	0.4		1.3		2.3		Berman & Chava (1999)
Microcystis aeruginosa[a]	80	0.6		0.8		2.5		Berman & Chava (1999)
Microcystis aeruginosa		0.34		0.31		0.41		C. M. Solomon (unpubl.)
Aphanizomenon ovalisporum[a]	80	0.6		0.4		1.3		Berman & Chava (1999)
RED LINEAGE								
Diatoms								
Thalassiosira pseudonana[a]	[b]	3.6		3.6		3.2		Ferguson et al. (1976)
Chaetoceros gracilis	170	1.84	0.02	1.56	0.10	1.28	0.06	Levasseur et al. (1993)
Chaetoceros gracilis	7	0.43	0.01	0.39	0.11	0.49	0.05	Levasseur et al. (1993)
Cyclotella sp.[a]	80	3.0		2.8		4.3		Berman & Chava (1999)
Pseudo-nitzschia multiseries, Pn-1[c]	150–20	0.75	0.1	0.75	0.18	0.3	0.1	Thessen et al. (2009)
Pseudo-nitzschia fraudulent, Pn10[c]	150–200	0.82	0.05	0.85	0.07	0.58	0.08	Thessen et al. (2009)
Pseudo-nitzschia callianth, Pn-13[c]	150–200	0.72	0.07	0.7	0.07	0.44	0.02	Thessen et al. (2009)
Dinoflagellates								
Gymnodinium sanguineum	170	0.41	0.01	0.43	0.01	0.18	0.03	Levasseur et al. (1993)
Gymnodinium sanguineum	7	0.17	0.02	0.14	0.02	0.1	0.02	Levasseur et al. (1993)
Karenia brevis strain C6	30	0.08	0.01	0.10	0.0	0.10	0.01	Sinclair et al. (2009)
Karenia brevis strain C3	30	0.09	0.01	0.10	0.01	0.10	0.01	Sinclair et al. (2009)
Karenia brevis strain CCMP 299	30	0.08	0.02	0.09	0.03	0.12	0.03	Sinclair et al. (2009)
Prorocentrum minimum	300	0.34	0.02	0.31	0.04	0.29	0.01	Solomon & Glibert (2008)
Karlodinium veneficum	300	0.42	0.06	0.52	0.06	0.49	0.07	Solomon & Glibert (2008)
Heterocapsa triquetra	300	0.21	0.04	0.24	0.0	0.23	0.01	Solomon & Glibert (2008)
Cryptophytes								
Storeatula major	300	0.65	0.02	0.71	0.03	0.69	0.02	Solomon & Glibert (2008)
Haptophytes								
Isochrysis sp.	300	0.85	0.01	0.72	0.01	0.78	0.0	Solomon & Glibert (2008)
Emiliania huxleyi strain 370	45–60	0.13	<0.01	0.15	<0.01	0.19	<0.01	Strom & Bright (2009)
Emiliania huxleyi strain 374 exp 1	45–60	0.21	<0.01	0.23	0.01	0.33	0.01	Strom & Bright (2009)
Emiliania huxleyi strain 373 exp 1	45–60	0.10	<0.01	0.05	0.02	0.09	<0.01	Strom & Bright (2009)
Emiliania huxleyi strain 379	45–60	0.18	0.01	0.15	0.02	0.07	<0.01	Strom & Bright (2009)
Raphidophytes								
Heterosigma akashiwo[d]	110	0.82	~0.02	0.89	~0.02	0.82	~0.03	Herndon & Cochlan (2007)
Heterosigma akashiwo[d]	40	0.46	~0.08	0.57	~0.08	0.61	~0.08	Herndon & Cochlan (2007)
Pelagophytes								
Aureococcus anophagefferens	400	0.55	0.03	nd		0.59	0.02	Pustizzi et al. (2004)
Aureococcus anophagefferens	100	0.45		nd		0.45		Pustizzi et al. (2004)
Aureococcus anophagefferens	50	0.51		0.48		0.59		Berg et al. (2008)
Aureoumbra lagunensis[d]	220	0.31	<0.01	0.68	0.04	0.55	0.03	Muhlstein & Villareal (2007)
Aureoumbra lagunensis[d]	140	0.26	<0.01	0.62	0.04	0.48	0.03	Muhlstein & Villareal (2007)
Aureoumbra lagunensis[d]	55	0.21	<0.01	0.41	0.03	0.45	0.02	Muhlstein & Villareal (2007)
GREEN LINEAGE								
Chlorophytes								
Dunaliella tertiolecta	170	1.41	0.09	1.42	0.04	1.33	0.06	Levasseur et al. (1993)
Dunaliella tertiolecta	7	0.25	0.01	0.27	0.02	0.3	0.02	Levasseur et al. (1993)
Pediastrum duplex[a]	80	0.9		0.7		1.8		Berman & Chava (1999)

[a]Growth rates were estimated from plotted changes during exponential growth
[b]Paper reported a light level of 5.4 klux for which no conversion is available to μmol photons m^{-2} s^{-1}
[c]This paper reports on the effect of N source for 9 strains of *Pseudo-nitzschia*; only the first clone for each *Pseudo-nitzschia* species is given here
[d]Error bars estimated from publication graph

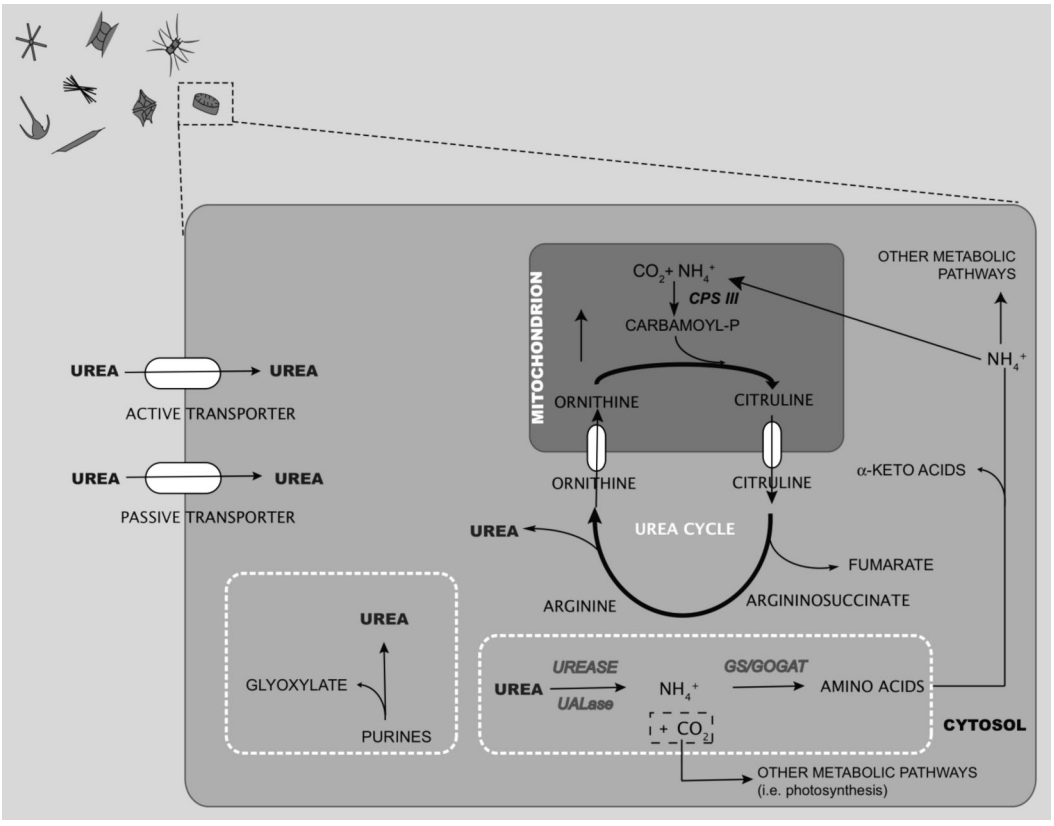

Fig. 1. Urea metabolism pathways. CPS III: carbamoylphosphate synthetase; GS/GOGAT: glutamine synthetase/glutamine: 2-oxoglutarate amidotransferase; UALase: urea amidolyase

and *Bacillus megaterium* (Jahns et al. 1988, Jahns & Kaltwasser 1989, Jahns 1992a,b), and genomic sequence data suggest that at least for *P. aeruginosa* the transporter is a *urt* homologue.

Genomic sequencing has revealed that marine unicellular photosynthetic eukaryotes possess the gene *DUR3* (Table 2), which encodes a high-affinity urea active transporter that is part of the larger sodium: solute symporter family (SSSF) of transport proteins (Wang et al. 2008). SSSF porters use the energy from a sodium gradient across the cell membrane to transport the solute into the cell (Jung 2002) and are also present in higher plants, mosses, and fungi (Wang et al. 2008). The *DUR* genes were initially identified as part of the allantoin catabolic pathway in yeast (Genbauffe & Cooper 1986) and also include the *DUR1* and *DUR2* genes which encode the urea carboxylase and allophanate hydrolase enzymes, respectively, discussed in further detail below. Of the phytoplankton *DUR3* transporters surveyed here, the red algal lineage *DUR3* amino acid sequences from the pelagophyte *Aureococcus anophagefferens*, the dinoflagellate *Heterocapsa triquetra,* and the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* group together. However, the *DUR3* sequences of the haptophyte *Emiliania huxleyi*, also of the red algal lineage, were highly divergent (Fig. 2). The green algal lineage phytoplankton *Ostreococcus* sp. *DUR3* clustered with the higher plants and the main red algal lineage group, while the freshwater *Chlamydomonas reinhardtii DUR3* sequences were more divergent. *A. anophagefferens* has one full-length *DUR3* gene and one truncated *DUR3* gene sequence in its genome (protein IDs 71789 and 6161, respectively), whereas *C. reinhardtii* has 3 closely related *DUR3* genes. The diatom *P. tricornutum* has 2 *DUR3* genes, whereas *T. pseudonana* has one *DUR3* gene. A literature review of urea uptake kinetics in phytoplankton cultures revealed that the maximum rate of uptake (V_{max}) may be higher in the red algal lineage phytoplankton, while affinity for urea (K_s) may be higher in the green lineage phytoplankton, suggesting a difference in enzyme kinetics for the different branches of the *DUR3* gene family (Table 3).

In addition to the high-affinity transporter *DUR3*, the movement of urea across cell membranes can be facilitated by families of proteins including (1) the urea/amide channels, (2) the major intrinsic proteins (MIPs), also known as water channels or aquaporins (AQPs), and (3) low-affinity urea transporters of the solute carrier family 14, homologous to mammalian kidney urea

Table 2. Urea metabolism genes in phytoplankton. A select number of freshwater and marine *Cyanobacteria*, chosen on the basis of prevalence in aquatic ecosystems, are represented in the table. Presence of genes in *Cyanobacteria* was determined by running BLAST searches to known genes at CyanoBase (http://genome.kazusa.or.jp/cyanobase/). Presence of genes in completely sequenced eukaryotic phytoplankton was determined first by doing searches in the Joint Genome Institute (JGI) database

Phytoplankton species	Urea transporter	Urea catabolism: Urease	Urea catabolism: Urea amidolyase (both urea carboxylase and allophanate hydrolase)
Protein domains	URT = IPR003439 DUR3 = IPR001734 SLC14A = IPR004937	IPR005848 (ureC, α subunit)	IPR005482 IPR000120
PHOTOSYNTHETIC BACTERIA			
Cyanophytes			
Synechocystis sp. *PCC 6803*	URT (*NP_442328*)	URE (*NP_440403*)	
Synechococcus sp. *WH8102*	URT (*NP_898527*)	URE (*NP_898538*)	
Nostoc sp. *PCC 7120* (formerly *Anabaena* sp. *PCC 7210*)	URT (*NP_485987*)	URE (*NP_487710*)	
Microcystis aeruginosa NIES-843	URT (*YP_001655632*)	URE (*YP_001661147*)	
Prochlorococcus marinus subsp. *pastoris* str. CMP1986 (formerly *Prochlorococcus marinus MED4*)	URT (*NP_893091*)	URE (*NP_893080*)	
Cyanothece sp. *ATCC 51142*	URT (*YP_001805935*)		
Trichodesmium erythraeum IMS101	URT (*YP_720094*)	URE (*YP_720654*)	
RED LINEAGE			
Diatoms			
Thalassiosira pseudonana (centric diatom; Armbrust et al. 2004)	DUR3 (*XP_002292926*) SLC14A	URE (*XP_002296690*)	
Phaeodactylum tricornutum (pennate diatom; Bowler et al. 2008)	DUR 3A-B (*XP_002180571*, *XP_002180573*) SLC14A	URE (*XP_002183086*)	
Haptophytes			
Emiliania huxleyi	DUR 3A-C (*PI#440179* [*isoA*], *460978* [*isoB*], *217311* [*isoC*]) SLC14A	URE (*PI#42377*)	
Pelagophytes			
Aureococcus anophagefferens	DUR3 (*PI#71789*)	URE (*PI#54430 and 77851*)	
GREEN LINEAGE			
Prasinophytes			
Micromonas pusilla (*CCMP 1545*)			
Micromonas sp. (*RCC299*)	DUR3 (*ACO64416*)	URE (*ACO69704*)	
Ostreococcus tauri	DUR3 (*CAL57595*)	URE (*CAL5759*)	
Ostreococcus lucimarinus	DUR3 (*XP_001416230*)	URE (*XP_001416478*)	
Chlorophytes			
Chlamydomonas reinhardtii	DUR3A-C (*XP_001702308* [*isoA*], *XP_001702309* [*isoB*], *XP00170417* [*isoC*])		DUR1 (*XP_001702324*) DUR2 (*XP_001702307*)

[a]OTC — Another name for the enzyme is ornithine carbamoyltransferase; [b]Sequence given by JGI for CPS for *Ostreococcus tauri* shows up as for *Ostreococcus lucimarinus* in GenBank

(http://genome.jgi-psf.org/), then BLAST searches to confirm homology (via identity%, scores, and e-values) to annotated genes in June 2009. Some enzymes have several subunits, but for consistency only genes with chosen protein domains (IPR) are listed. Accession numbers or Protein ID (PI#) are provided in italics. An empty box does not necessarily indicate absence of the gene; possibly the gene has not yet been annotated in the relevant database

Urea cycle					Purine catabolism	
Carbamoyl-phosphate synthetase	Ornithine transcarb-amylase[a]	Arginase	Argino-succinate lyase	Argino-succinate synthetase	Purine transporter (xanthinine uracil permease)	Guanine deaminase
IPR005480	IPR002292	IPR006035	IPR000362	IPR001518	IPR000643	IPR006680
CPS (*BAA10403*)	OTC (*NP_442776*)	ARG (*NP_440030*)				
CPS (*NP_896923*)	OTC (*NP_897679*)	ARG (*NP_898511*)				
CPS (*NP_487849*)	OTC (*NP_488947*)					
CPS (*YP_001660056*)	OTC (*YP_001660424*)	ARG (*YP_001659724*) (*YP_001659732*)				
CPS (*NP_892943*)	OTC (*NP_893380*)	ARG (*NP_893803*)				
CPS (*YP_001803454*)	OTC (*YP_001804665*)					
CPS (*YP_722523*)	OTC (*YP_721117*)					
CPS (*XP_002289336*)	OTC (*XP_002286586*)	ARG (*XP_002296117*)	ASL (*XP_002291698*)	ASS (*XP_002295518*)	URA (*XP_002295239*)	
CPS (*XP_002183539*)	OTC (*XP_002184453*)	ARG (*XP_002182650*)	ASL (*XP_002178853*)	ASS (*XP_002185721*)	URA (*XP_002185427*)	GDA (*XP_002178558*)
CPS (*PI#422007*)	OTC (*PI#236460*)	ARG (*PI#434747*)	ASL (*PI#456474*)	ASS (*PI#441299*)	URA (*PI#203630*)	GDA (*PI#42202* and *42244*)
CPS (*PI#38679*)	OTC (*PI#33293*)	ARG (*PI#77903*)	ASL (*PI#32664*)	ASS (*PI#26092*)	URA (*PI#55502*)	GDA (*PI#70268*)
CPS (*EEH57359*)	OTC (*EEH59886*)	ARG (*EEH57601*)	ASL (*EH60517*)	ASS (*EEH52445*)		
CPS (*ACO62638*)	OTC (*ACO68586*)	ARG (*ACO62762*)	ASL (*ACO61144*)	ASS (*ACO67580*)	URA (*ACO69126*)	
CPS (*XP_001419372*)[b]	OTC (*CAL50057*)	ARG (*CAL54440*)	ASL (*CAL57392*)	ASS (*CAL58290*)		
CPS (*XP_001419372*)	OTC (*XP_001415432*)	ARG (*XP_001417251*)	ASL (*XP_001420706*)	ASS (*XP_001421662*)		
CPS (*XP_001691359*)	OTC (*XP_001690929*)	ARG (*XP_001702430*)	ASL (*XP_001689515*)	ASS (*XP_001696749*)	URA (*XP_001702660*)	GDA (*XP_001695623*)

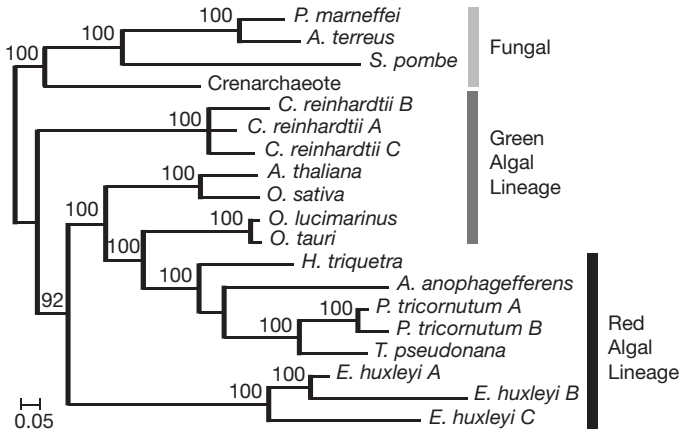

Fig. 2. Unrooted phylogenetic tree of DUR3 protein sequences (approx. 700 amino acids) aligned with the CLUSTAL W multiple sequence alignment algorithm (Thompson et al. 1994) and constructed with MrBayes 3.1 (Ronquist & Huelsenbeck 2003). The scale bar shows number of substitutions per site. The protein sequence of *Heterocapsa triquetra* was created from 4 separate expressed sequence tag (EST) sequences deposited in GenBank. Dark shading indicates red algal lineages, medium gray shading indicates green algal lineages, and light gray shading represents fungal sequences and the Crenarchaeote sequence. Full scientific names are *Arabidopsis thaliana, Aspergillus terreus, Aureococcus anophagefferens, Chlamydomonas reinhardtii, Emiliania huxleyi, Heterocapsa triquetra, Ostreococcus lucimarinus, Oryza sativa, Ostreococcus tauri, Penicillium marneffei, Phaeodactylum tricornutum, Schizosaccharomyces pombe* and *Thalassiosira pseudonana*

transporters (Raunser et al. 2009). In vascular plants, urea uptake is thought primarily to occur through the high-affinity transporter *DUR3,* but when urea concentrations are high, low-affinity transporters such as MIPs may become more important (Wang et al. 2008). Sequence similarity searches have revealed that some marine phytoplankton genomes contain urea transporter (SLC14A) homologues. *Emiliania huxleyi* has one, and *Thalassiosira pseudonana* has 2 urea transporter-type transporters (Table 2). Many pathogenic bacteria have urea belonging to the urea/amide or urea transporter/SLC14A families (Raunser et al. 2009), and basic local alignment search tool (BLAST) searches suggest that urea transporter homologues might also be present in a variety of nonpathogenic bacteria (results not shown), although their function remains to be proven. Berg et al. (2008) also suggested that *AaNAR1.3* might be a urea transporter in phytoplankton, as expression of this gene was higher in *Aureococcus* cells grown on urea than in any other N source. In bacteria, NAR1.3 has been characterized as a formate/ NO_2^- transporter, but it has a different and not well-understood function in the green alga *Chlamydomonas reinhardtii,* where it does not transport NO_2^- or NO_3^- (Mariscal et al. 2006).

In higher plants, urea is metabolized quickly and does not accumulate in the cytosol. If urea were stored in the cell, then intracellular urea transporters would be essential for moving urea accumulating in the cytosol into storage vacuoles, in order to prevent possible toxicity to the cell, but there are no reports of any vacuolar urea concentrations in higher plants nor channel-like active tonoplast/vacuolar transporters (Wang et al. 2008). In contrast to higher plants, cellular accumulation of urea up to 280 mM-N occurs in some dinoflagellates such as *Prorocentrum minimum* and *Karlodinium veneficum* (Solomon & Glibert 2008). Determining the urea storage mechanisms used by organisms that can accumulate urea would be an interesting avenue of research and would be aided by the generation of many more sequence data for dinoflagellates.

Regulation of urea uptake

Urea transporters, like those described above, bring new N (that is, N that can contribute to increases in biomass) into the microbial cell, so it is not surprising that culture studies have shown that urea uptake and expression of UTs in bacteria and phytoplankton are influenced by the availability of alternative N substrates, especially NH_4^+. In *Corynebacterium glutamicum*, expression of the UT (*urt*) genes increases when growth is limited by N supply (Silberbach & Burkovski 2006), and in *Deleya* (now *Halomonas*) *venusta* HG1, urea uptake was lowest in cells grown on NH_4^+ and highest in cells grown on NO_3^- and in cells deprived of N (Jahns 1992b). Phytoplankton, particularly diatoms, grown under N-starved or N-replete conditions consistently have shown decreased rates of urea uptake after the addition of NH_4^+ and/or NO_3^- to cultures (Rees & Syrett 1979, Lund 1987, Lomas 2004, Jauzein et al. 2008a). For example, in the N-starved *Phaeodactylum tricornutum*, urea uptake was inhibited after transfer to a medium containing NH_4^+ (Rees & Syrett 1979, Molloy & Syrett 1988), and in *Skeletonema costatum*, Lund (1987) observed a decrease of from 82 to 84 % in urea uptake 3 h after the addition of either 10 µM-N NO_3^- or NH_4^+. As in these diatoms, urea uptake in dinoflagellates may be inhibited by inorganic N substrates. In a comparison of different strains of the dinoflagellate *Alexandrium catenella*, strong inhibition of urea uptake by NH_4^+ was observed in only one strain, which may be explained by the adaptation of that strain to a low NH_4^+ and high urea environment as compared to the other strains (Jauzein et al. 2008b).

Similar observations of the influence of NH_4^+ on rates of urea uptake have been made in many field studies. Urea uptake rates generally decrease after the addition of NH_4^+ or with increasing ambient NH_4^+ con-

Table 3 (and overleaf). Urea uptake kinetics (K_s, V_{max}, and ρ_{max}) in select bacterial and phytoplankton species grown on urea in culture. Additional information about type of urea transporters and inhibitors tested (chlorophenylhydrazone, CCCP; N,N′-dicyclohexylcarbodiimide, DCCD; and 3-[3,4-dichlorophenyl]-1,1-dimethylurea, DCMU) as well as the physiological state of the cells is provided. CCCP, DCCD, and DCMU are inhibitors of different pathways involved with photosynthesis

Species	Type of urea transporter/notes about inhibitors	State of cells	Half-saturation constant, K_s (µM-N)	Maximum uptake rate, V_{max} (h^{-1})	Maximum transport rate, ρ_{max} (µM-N h^{-1})	Source
HETEROTROPHIC BACTERIA						
Deleya venusta HG1	Azide, cyanide, CCCP completely inhibited uptake; DCCD had a minor effect; uptake increased with increasing NaCl	N-replete (harvested at endof exponential growth), washed in N-free media; then urea was added	1.4		0.234[a]	Jahns (1992b)
Corynebacterium glutamicum	CCCP inhibited uptake; presence of valinomycin and K^+ decreased uptake; concluded that a proton-motive-force-linked transport system was present	N-replete	9		0.12–21[b]	Siewe et al. (1998)
MEDIAN			**5.2**		**10.6**	
PHOTOSYNTHETIC BACTERIA						
Cyanophytes						
Pseudoanabaena catenata	Increasing amounts of CCCP (>10 µM) decreased uptake	N-replete, then re-suspended in N-free media, then urea was added	0.4			Healey (1977)
PHYTOPLANKTON						
RED LINEAGE						
Diatoms						
Cyclotella nana		NO_2^- depleted cultures (but not of other nutrients), then urea was added	0.41	0.0076		McCarthy (1972)
Ditylum brightwellii		NO_2^- depleted cultures (but not of other nutrients), then urea was added	0.42	0.1090		McCarthy (1972)
Melosira italica	Cyanide inhibited 75.3 and 100% in light and darkness, respectively; 2.4-dinitrophenol had an inhibitory effect	N-starved cultures	11.22 (light) 65.07 (dark)		1.11 (light) 2.18 (dark)	Cimbleris & Cáceres (1991)
Phaeodactylum tricornutum	CCCP inhibited uptake, but DCMU did not	Both N-replete and N-starved cultures and harvested after 48 h of exponential growth	0.57 ± 0.16 (N-replete cells) 1.0 ± 0.22 7.60 (N-starved cells)		6	Rees & Syrett (1979)

Table 3 (continued)

Species	Type of urea transporter/notes about inhibitors	State of cells	Half-saturation constant, K_s (µM-N)	Maximum uptake rate, V_{max} (h^{-1})	Maximum transport rate, ρ_{max} (µM-N h^{-1})	Source
Pseudo-nitzschia australis	Did not exhibit saturation over range of 0–40 µM-N					Cochlan et al. (2008)
Skeletonema costatum		NO_2^- depleted cultures (but not of other nutrients), then urea was added	1.41	0.0151		McCarthy (1972)
Thalassiosira fluviatilis		NO_2^- depleted cultures (but not of other nutrients), then urea was added	1.66 0.45	0.0239 0.0303		McCarthy (1972)
Dinoflagellates						
Alexandrium catenella ACTO3 (French clone)		N-replete cultures	0.6–2.3	0.004–0.023		Jauzein et al. (2008b)
Alexandrium catenella ACT 2000		N-replete, then re-suspended in N-free media, then urea was added	28.4 ± 15.0	0.025 ± 0.008		Collos et al. (2004)
Karenia brevis (average for 3 strains)		N-starved cultures, then urea was added	2.37 (diurnal) 0.48 (nocturnal)		0.71 (diurnal) 0.51 (nocturnal)	Sinclair et al. (2009)
Prorocentrum minimum		N-replete cultures; mid-exponential phase	1.82		152.8 fmol $cell^{-1}$ h^{-1} [c]	Fan et al. (2003b)
Raphidophytes						
Heterosigma akashiwo		NO_3^- replete but not N-starved cultures; exponential phase	0.42	0.00289		Herndon & Cochlan (2007)
MEDIAN			**1.21**	**0.023**	**1.645**	
GREEN LINEAGE						
Chlorophytes						
Chlorella	DCMU inhibited uptake	N-replete, then re-suspended in N-free media, then urea was added	16.5 ± 4.8		147	Bekheet & Syrett (1979)
Chlorella reinhardtii		N-replete; exponential growth	0.87	0.0074		Kirk & Kirk (1978)
Eudorina elegans		N-replete; exponential growth	0.14	0.0023		Kirk & Kirk (1978)
Gonium pectorale		N-replete; exponential growth	0.14	0.0014		Kirk & Kirk (1978)
Pandorina morum		N-replete; exponential growth	2.6	0.0076		Kirk & Kirk (1978)
Pleodorina californica		N-replete; exponential growth	0.47	0.0066		Kirk & Kirk (1978)
Scenedesmus quadricauda	Increasing amounts of CCCP decreased uptake	N-replete, then re-suspended in N-free media, then urea was added	1.2			Healey (1977)
Volvox carteri		N-replete; exponential growth	0.85	0.0108		Kirk & Kirk (1978)
MEDIAN			**0.86**	**0.007**		

[a]Converted from nmol urea (mg protein)$^{-1}$ min^{-1} or pmol h^{-1} mg $protein^{-1}$
[b]Converted from nmol min^{-1} (mg dry weight)$^{-1}$
[c]Concentration of cells (cells l^{-1}) was not provided in paper, to permit conversion to µM-N h^{-1}

centrations. For example, urea uptake rates decreased after the addition of 20 µM-N NH_4^+ but not after the addition of 20 µM-N NO_3^- in field incubations of Baltic seawater (Tamminen & Irmisch 1996). Urea uptake was found to be inhibited or repressed by NH_4^+ at concentrations higher than 1 to 2 µM-N in Oslofjord, Norway (Kristiansen 1983), 40 µM-N in the Neuse Estuary, NC (Twomey et al. 2005), and 5 µM-N in the Chesapeake Bay (Solomon 2006; Fig. 3).

Alteration of transporter activity is the principal mechanism underlying the broadly observed repression of urea uptake by NH_4^+ in bacteria and phytoplankton in culture and natural plankton communities (e.g. Rees & Syrett 1979, Lund 1987, Lomas 2004, Jauzein et al. 2008a). For example, Berg et al. (2008) found that the expression of the urea active transporter gene (*AaDUR3*) in *Aureococcus anophagefferens* was 20- to 50-fold higher in cultures grown on urea compared with NO_3^- and NH_4^+, respectively. In *Corynebacterium glutamicum,* expression of the *urt* genes is controlled by N availability via the transcription regulator AmtR (Silberbach & Burkovski 2006), and in *Cyanobacteria* as diverse as *Anabaena* and *Prochlorococcus* the *urt* genes are regulated by N availability via the global N transcription regulator NtcA (see below; Valladares et al. 2002, Su et al. 2005, Tolonen et al. 2006). More studies are needed to investigate differences in regulation and kinetics of urea uptake among different bacterial and phytoplankton taxa and strains from a variety of environments with varying N concentrations (Burkholder & Glibert 2006, 2009).

Another environmental factor that may influence rates of urea uptake is irradiance. In typical aquatic ecosystems, most urea uptake occurs via active membrane transporters that require energy. The energy for urea uptake may be obtained from photophosphorylation in phytoplankton during the day (Rees & Syrett 1979, Siewe et al. 1998, Beckers et al. 2004) and from oxidative phosphorylation in heterotrophs or phytoplankton during the night (Cimbleris & Cáceres 1991). In Chesapeake Bay plankton, a diel pattern in urea uptake was observed with the highest rates of urea uptake in mid-afternoon and the lowest rates during the night (Bronk et al. 1998). However, urea uptake can take place during the dark in dinoflagellate-dominated assemblages (Kudela & Cochlan 2000, Fan & Glibert 2005, Sinclair et al. 2009). In the Chesapeake Bay plume, urea uptake represented a larger proportion of total N utilization during the night than during the day, because utilization of NO_3^- and NH_4^+ decreased more at night (Glibert et al. 1991). Urea uptake increased with increasing irradiance during a *Prorocentrum minimum* bloom in the Choptank River in spite of the fact that urea uptake in this species is not light-dependent on short time scales (30 min; Fan & Glibert 2005). This observation may indicate that the

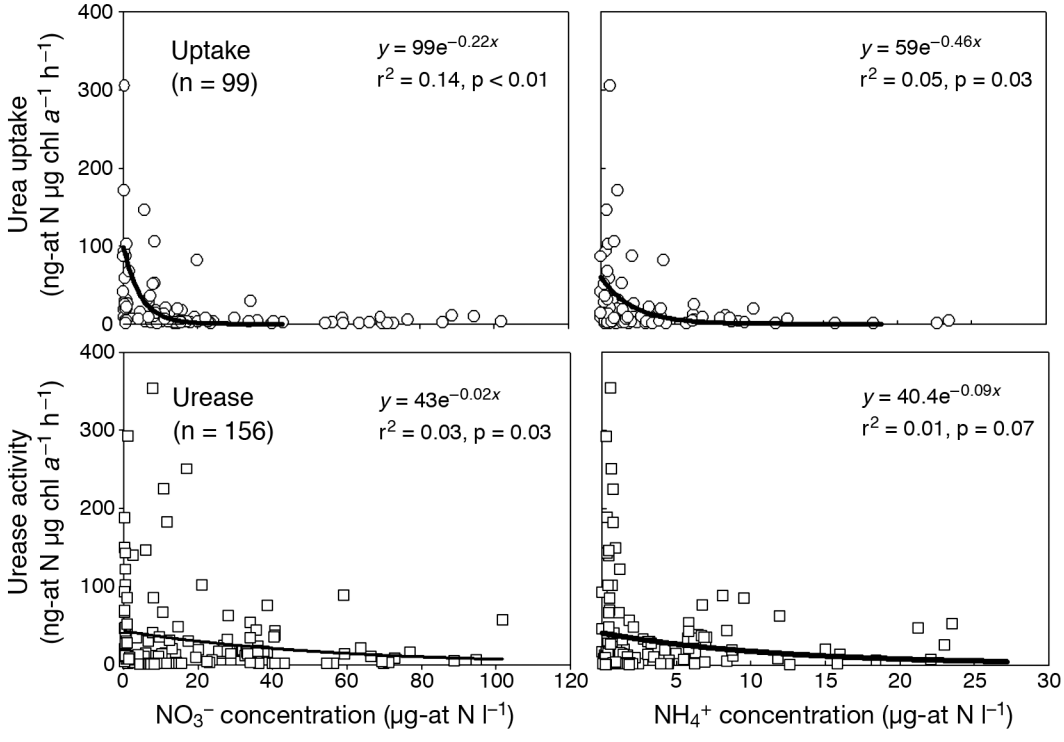

Fig. 3. Urea uptake or urease activity as a function of NO_3^- concentration and NH_4^+ concentration in the Chesapeake Bay, USA (from Solomon 2006)

capacity to convert N from urea that is taken up into amino acids and other components of cell biomass, which is likely to be light-dependent in phytoplankton, may also affect the rate of urea uptake. It is not yet clear how urea uptake rates are regulated by irradiance in different phytoplankton taxonomic groups.

Field studies have shown that maximum urea uptake rates occur during the summer, while minimum rates occur during the winter in various temperate estuaries (Kristiansen 1983, Glibert et al. 1991, Bronk et al. 1998, Lomas et al. 2002, Solomon 2006), which may reflect a direct effect of temperature on urea uptake, seasonal changes in plankton community composition, and lastly a seasonal decrease in microbial urea production leading to decreased availability in winter. In contrast to NO_3^-, NH_4^+ and urea uptake rates have a positive relationship with temperature (between 5 and 30°C) in diatom-dominated assemblages (Lomas & Glibert 1999a). Urea uptake increased as a function of temperature in the diatom *Melosira italica*, with a Q_{10} of 1.94 (Cimbleris & Cáceres 1991). However, urea uptake remained constant over a narrower temperature range of 10 to 25°C for *Prorocentrum minimum* in the Chesapeake Bay and Neuse Estuary (Fan et al. 2003b). These findings suggest that the relationship between temperature and urea uptake may be species-dependent.

The urea cycle and purine catabolism

In addition to uptake of external urea into the cell, urea is produced intracellularly in most organisms by the urea cycle and/or by purine catabolism (Vogels & Van der Drift 1976, Antia et al. 1991, Allen et al. 2006, Berg & Jørgensen 2006, Wang et al. 2008). The first step in the utilization of purines as a N source involves the deamination of guanine to xanthine, or the deamination of adenine to hypoxanthinine, followed by the conversion of hypoxanthine to xanthine (Vogels & Van der Drift 1976). Xanthine is then converted into uric acid by the enzyme xanthine oxidase. Uric acid is further broken down into ureides such as allantoin and allantoic acid by the enzymes uricase, allantoinase, and allantoicase (allantoate amidinohydrolase), producing urea (Vogels & Van der Drift 1976, Garrett & Grisham 1995, Wang et al. 2008). Interestingly, guanine appears to be more widely produced, leading to a higher availability, and therefore higher rates of uptake and catabolism, compared with adenine in the marine environment (Antia et al. 1975, Shah & Syrett 1982, 1984, Berman et al. 1999, Berg & Jørgensen 2006). On the basis of sequence similarity, a putative purine transporter gene, *AaURA*, was discovered in *Aureococcus anophagefferens* and shown to be highly expressed during growth on a number of different N sources and under N-limited conditions (Berg et al. 2008). The enzyme guanine deaminase (but not adenine deaminase) has also been identified on the basis of sequence similarity in *A. anophagefferens*, raising the possibility that *AaURA* may be specific for guanine or its derivative xanthine. The sequence of *AaURA* is similar to the xanthine uracil permease and the purine permease identified in the genomes of *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, respectively. As with *A. anophagefferens*, these 2 diatoms also have homologues of the guanine deaminase enzyme (Table 2).

Urea is also produced intracellularly by the activity of arginase, which converts arginine into ornithine (Vogels & Van der Drift 1976, Antia et al. 1991, Allen et al. 2006, Wang et al. 2008; Fig. 1). In eukaryotic phytoplankton, arginase may function as part of a complete urea cycle, since a complete set of urea cycle genes has been identified in the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* as well as the pelagophyte *Aureococcus anophagefferens* and the haptophyte *Emiliania huxleyi* (Table 2). *Cyanobacteria* appear not to have a complete set of the urea cycle genes in their genomes but do contain some genes such as carbamoylphosphate synthetase and arginase (Table 2). It is currently unknown if dinoflagellates contain any urea cycle genes, as no member of this group has been sequenced to date and the relevant biochemical assays have not been performed. The activity of urea cycle enzymes has been demonstrated in bioassays with estuarine water in which the addition of amino acids or urea cycle intermediates resulted in release of urea (Berman et al. 1999, Jørgensen et al. 1999a).

The role of the urea cycle in phytoplankton metabolism and evolution is currently being discussed and investigated. The long-known function of the urea cycle in animals is to excrete excess N produced by α-keto amino acid catabolism (e.g. glutamate, proline, arginine, and histidine; Garrett & Grisham 1995, Parker et al. 2008). The discovery of the urea cycle genes in the diatom *Thalassiosira pseudonana*, the first eukaryotic phytoplankton genome to be sequenced, was a surprise because urea is not considered as a 'waste' product in those organisms and the NH_4^+ resulting from urea catabolism is returned back to anabolic pathways that form glutamine and glutamate (Armbrust et al. 2004, Vardi et al. 2008). The urea produced via the urea cycle, in addition to regulating amino acid catabolism, may serve as an osmolyte for the cell (Armbrust et al. 2004, Allen et al. 2006). Another hypothesis about the role of the urea cycle in diatoms is that intermediates from the urea cycle contribute to other aspects of cell metabolism. For instance, ornithine is used to make spermine and spermidine, which have roles in

silica precipitation (Armbrust et al. 2004). Phylogenic analysis of *Phaeodactylum tricornutum* revealed that genes involved in the urea cycle seem to have a prokaryotic origin, which suggests that those genes could have been acquired via horizontal gene transfer, but by which possible mechanism (e.g. viral infection, phagotrophy, association with organelles, or with intracellular endosymbionts) is not clear (Parker et al. 2008, Vardi et al. 2008).

Intracellular catabolism of urea

Urea, from either internal or external sources, can be broken down into NH_4^+ and inorganic carbon (C) by urease (Mobley & Hausinger 1989, Antia et al. 1991, Zehr & Ward 2002) or by UALase (Antia et al. 1991, Hausinger 2004). Urease is by far the better-characterized enzyme of the two. Urease is a cytosolic enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate. Carbamate spontaneously decomposes to yield a second molecule of ammonia and carbonic acid (Mobley & Hausinger 1989, Mobley et al. 1995):

$$CO\text{-}(NH_2)_2 + H_2O \rightarrow NH_3 + OH\text{-}CO\text{-}NH_2$$
$$OH\text{-}CO\text{-}NH_2 + H_2O \rightarrow NH_3 + H_2CO_3$$

At environmental pH (~7–8), the ammonia molecule equilibrates with water and becomes protonated, forming NH_4^+. The cell then uses the NH_4^+ produced by catabolism of urea for protein synthesis (Wheeler 1983, Capone 2000).

In most bacteria, including *Cyanobacteria*, the urease enzyme occurs as 2 small (gamma, γ, and beta, β), and one large (alpha, α) protein subunits encoded by the *ureA*, *ureB,* and *ureC* genes, respectively (Mobley et al. 1995). In the bacterium *Helicobacter pylori,* the γ and β subunits are fused into one protein, and the complete urease enzyme therefore has 2 subunits. In eukaryotes, all 3 subunits are fused into a single protein, encoded by the *ureABC* gene, where the N terminus is homologous to the bacterial γ subunit, the internal sequence is homologous to the β subunit, and the C terminus is homologous to the bacterial α subunit. There are also several accessory genes (*ureD*, *ureE*, *ureF*, and *ureG*) that aid in the production of active urease by supporting the assembly of the nickel metallocenter of the enzyme (Mobley et al. 1995).

Urease genes have been found in the recently sequenced red lineage marine phytoplankton genomes, including *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, *Emiliana huxleyi*, and *Aureococcus anophagefferens* as well as in several green lineage marine phytoplankton genomes including 2 *Ostreococcus* and 1 *Micromonas* strain (RCC299; Table 2). The urease protein sequences of the red-lineage phytoplankton cluster together, whereas the green-lineage phytoplankton cluster more closely with higher plants (Fig. 4). A fragment of the *ureABC* gene has also been amplified from the diatom *Thalassiosira oceanica*, 2 prymnesiophytes (*Pseudoisochrysis paradoxa* and the unidentified species CCMP 1249), and the eustigmatophyte *Nannochloropsis gaditana* using primers for 2 highly conserved motifs in the *ureC* region (Baker et al. 2009). The number, location, and length of introns in the *ureABC* gene of phytoplankton vary but are more similar among more closely related taxa. *A. anophagefferens* has 3 similar (less than 5% different) *ureABC* gene loci in its genome, though one of these (Protein ID 72133) appears to be truncated (missing the N-terminal region; Table 2). In contrast, the diatoms *P. tricornutum* and *T. pseudonana*, the haptophyte *E. huxleyi,* and other marine phytoplankton genomes sequenced to date each have only one *ureABC* gene (Baker et al. 2009; Table 2).

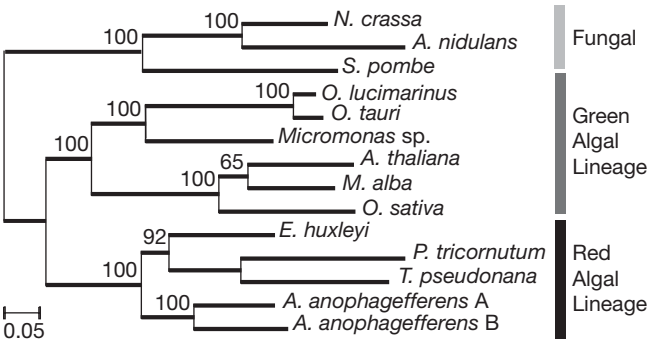

Fig. 4. Unrooted phylogenetic tree of urease protein sequences (approx. 830 amino acids) aligned with the CLUSTAL W multiple sequence alignment algorithm (Thompson et al. 1994) and constructed with MrBayes 3.1 (Ronquist & Huelsenbeck 2003). The scale bar shows number of substitutions per site. Shading as in Fig. 2. Full scientific names are *Arabidopsis thaliana, Aspergillus nidulans, Aureococcus anophagefferens, Emiliania huxleyi, Morus alba, Neurospora crassa, Ostreococcus lucimarinus, Oryza sativa, Ostreococcus tauri, Phaeodactylum tricornutum, Schizosaccharomyces pombe* and *Thalassiosira pseudonana*

Urease genes were found by protein-protein BLAST in about one third (454 of 1331) of the prokaryotic genomes available in GenBank (last searched December 2008; Table 4). Urease may be absent from some major groups of bacteria (e.g. *Spirochaetes*), although sequencing of additional genomes may reveal its presence in at least some members. In other groups, urease may be restricted to one or a few genera or families (e.g. among the *Bacteroidetes*, *Chlamydiae*, *Tenericutes*, and *Archaea*), while in some groups urease is much more common and widespread (e.g. among the *Actinobacteria*, *Cyanobacteria*, and *Proteobacteria*). Most ureolytic bacteria have only one copy of the *ure*

Table 4. Distribution of urease among sequenced prokaryotic genomes. Values are the percent of genomes, species, or genera in each group that contain a urease gene (determined by protein-protein BLAST); values in parentheses are the number with urease/total number of genomes. Data from GenBank, last searched December 2008

Group	% urease genome	% urease species	% urease genus
BACTERIA			
Actinobacteria[f]	59 (42/71)	58 (33/57)	46 (12/26)
Aquificae	0 (0/6)	0 (0/6)	0 (0/5)
Bacteroidetes/Chlorobi[a]	4 (2/56)	4 (2/51)	7 (2/27)
Chlamydiae/Verrucomicrobia[b]	10 (2/21)	13 (2/15)	10 (1/10)
Chloroflexi	18 (2/11)	18 (2/11)	40 (2/5)
Cyanobacteria	73 (35/48)	79 (11/14)	80 (12/15)
Deinococcus-Thermus	20 (1/5)	25 (1/4)	50 (1/2)
Dictyoglomi	0 (0/1)	0 (0/1)	0 (0/1)
Fibrobacteres/Acidobacteria	0 (0/5)	0 (0/5)	0 (0/4)
Firmicutes	15 (40/272)	17 (22/127)	24 (11/46)
Fusobacteria	0 (0/3)	0 (0/1)	0 (0/1)
Nitrospirae	0 (0/1)	0 (0/1)	0 (0/1)
Planctomycetes	0 (0/3)	0 (0/3)	0 (0/3)
Alphaproteobacteria[f]	51 (71/139)	52 (55/105)	54 (32/59)
Betaproteobacteria[f]	81 (83/103)	75 (38/51)	69 (18/26)
Gammaproteobacteria[f]	37 (118/316)	38 (53/140)	50 (35/70)
Delta/epsilonproteobacteria[f]	23 (14/62)	15 (6/40)	18 (4/22)
Other *Proteobacteria*	50 (1/2)	50 (1/2)	50 (1/2)
Spirochaetes	0 (0/32)	0 (0/15)	0 (0/3)
Tenericutes[c]	39 (14/36)	10 (2/21)	13 (1/8)
Thermodesulfobacteria	0 (0/1)	0 (0/1)	0 (0/1)
Thermotogae	0 (0/8)	0 (0/7)	0 (0/4)
ARCHEA			
Crenarchaeota[d]	6 (1/16)	6 (1/16)	9 (1/11)
Euryarchaeota[e]	11 (4/38)	12 (4/34)	16 (4/25)
Other *Archaea*	0 (0/2)	0 (0/2)	0 (0/2)

[a]Only the genera *Flavobacterium* and *Cytophaga* have urease
[b]Only the *Opitutaceae* have urease
[c]Only the genus *Ureaplasma* has urease
[d]Only the genus *Sulfolobus* has urease
[e]Only some of the *Halobacteriaceae* have urease
[f]Some members of these groups have more than one urease gene

genes encoding urease, but some *Proteobacteria* and *Actinobacteria* have 2 or 3 copies. The multiple copies in a single bacterial genome are generally very different from one another (~70% nucleotide sequence identity), and whether they have distinct biochemical properties or are regulated differently remains to be investigated. Despite the large number of bacterial urease sequences (and rapidly growing number of eukaryotic urease sequences) now available, the organisms from which more than half of the urease sequences recovered directly from estuarine and open ocean seawater samples arose cannot yet be identified (Collier et al. 2009), suggesting that there is still a great deal to learn about which organisms are responsible for urea degradation in aquatic ecosystems.

The alternative pathway for urea degradation occurs via ATP: urea amidolyase (UALase; EC 3.5.1.45; Antia et al. 1991, Hausinger 2004). UALase occurs either as a fusion of urea carboxylase (EC 6.3.4) and allophanate hydrolase (EC 3.5.1.54) proteins, or as 2 separate proteins that work together to degrade urea. In the yeast *Saccharomyces cerevisiae*, UALase is a single, fused protein encoded by the *DUR1,2* gene, whereas in the green alga *Chlamydomonas reinhardtii*, which is the only sequenced eukaryotic phototroph possessing UALase, the 2 activities occur in separate proteins encoded by the *DUR1* and *DUR2* genes (Table 2). The ATP-dependent urea carboxylase protein (located at the C-terminus of the fusion yeast protein) catalyzes the first reaction:

$$CO\text{-}(NH_2)_2 + HCO_3^- + ATP \rightarrow (NH_2)\text{-}CO\text{-}NH\text{-}COO^- + H_2O + ADP + P_i$$

whereas the allophanate hydrolase (homologous to the N-terminal domain of the yeast protein) catalyzes the second reaction:

$$(NH_2)\text{-}CO\text{-}NH\text{-}COO^- + 3H_2O + H^+ \rightarrow 2NH_4^+ + 2HCO_3^-$$

The UALase homologue (URC3,5) of *Saccharomyces kluyveri* has recently been characterized as an integral component of a novel pyrimidine catabolic pathway in which urea is an intermediate (Andersen et al. 2008). *DUR 1,2* is also highly active on the amides acetamide and formamide (Roon & Levenberg 1972, Kanamori et al. 2004).

Kanamori et al. (2004) reported segregated urea carboxylase and allophanate hydrolase enzyme activities in the alpha proteobacterium *Oleomonas sagaranensis*. A search of GenBank for proteins with the same predicted conserved domain structure as the urea carboxylases of *Chlamydomonas* and *O. sagaranensis* (using the Conserved Domain Architecture Retrieval Tool) revealed putative urea carboxylases in a variety of bacteria, mostly among the *Proteobacteria* and *Actinobacteria* (data not shown). This result suggests that the UALase pathway may be present in a variety of bacteria, some of which also have urease and some of which do not. The presence of UALase in bacteria dramatically broadens the taxonomic distribution of this pathway, previously thought to be restricted to some yeasts and chlorophytes (Hausinger 2004). Much more biochemical and physiological work will be required to determine what role UALase might play in the N metabolism of planktonic bacteria.

Regulation of urea catabolism

In phytoplankton that have urease, a basal level of urease activity is usually always detectable, but the level of activity varies with N source (Antia et al. 1991, Collier et al. 1999, Peers et al. 2000, Dyhrman & Anderson 2003, Lomas 2004, Solomon & Glibert 2008), suggesting that urease activity is regulated by external or internal factors, as is urea uptake. However, the pattern of regulation may differ not only among but also within phytoplankton taxonomic groups (Fig. 5). In some organisms, lower urease activity is found in NH_4^+-grown cultures than in NO_3^-- and urea-grown cultures, as in the case of the dinoflagellates *Prorocentrum minimum* and *Karlodinium veneficum* (Fan et al. 2003a, Solomon & Glibert 2008). In these organisms, as in many *Cyanobacteria*, when insufficient NH_4^+ is available to meet N demand, urease activity may increase in response to limitation of inorganic N. In contrast, urease activity in the dinoflagellate *Alexandrium fundyense* was the highest in N-starved and urea-grown cultures, lower in NH_4^+, and not detected in a NO_3^--grown culture (Dyhrman & Anderson 2003), while in *Heterocapsa triquetra*, urease activity was higher when grown on NH_4^+ than on NO_3^- or urea (Solomon & Glibert 2008). In these organisms, urease activity may be more strongly influenced by factors such as the size of the intracellular urea pool, which could change with variations in rates of urea uptake or production of urea from urea cycle activity or amino acid and purine catabolism. Differences in regulation can even be found among closely related organisms. For example, urease activity was the same regardless of N source in one clone of the diatom *Thalassiosira weissflogii* (Peers et al. 2000), but down-regulated in another clone when grown on NO_3^- (Fan et al. 2003a, Lomas 2004). In field studies with natural phytoplankton communities, urease activity appears to be inversely related to concentrations of NH_4^+ and NO_3^- (Fig. 3).

Although the presence of NH_4^+ may inhibit the use of urea in many organisms, the mechanism of NH_4^+ repression (also called N control) is best understood for *Cyanobacteria*. In *Cyanobacteria*, the expression of *ure* genes (as well as *urt* genes and the genes required to use a variety of other N sources) is generally regulated by cellular N status via the DNA-binding protein NtcA and the concentration of 2-oxoglutarate (Lindell & Post 2001, Flores et al. 2005, Muro-Pastor et al. 2005, Espinosa et al. 2006). The compound 2-oxoglutarate is the C substrate used by glutamate synthetase to incorporate N into new amino acids, and because that is its major metabolic role, the concentration of 2-oxoglutarate measures the balance between C and N assimilation in *Cyanobacteria*. Within this general regulatory outline, there are differences among *Cyanobacteria*. For example, *Synechococcus* WH7805 had 2-fold lower urease activity when grown on NH_4^+ compared to urea, but *Synechococcus* WH8112 showed no difference in urease activity on NH_4^+ versus urea. Both strains had much higher urease activity when grown on NO_3^- than on urea (J. L. Collier unpubl. data). Similarly, urease expression increased in response to N deprivation in both of the *Prochlorococcus* strains studied by Tolonen et al. (2006), but there were other differences in N regulation between them. Urease is not under the control of NtcA in all *Cyanobacteria*. Instead, it appears to be expressed constitutively in *Cyanobacteria* that can accumulate cyanophycin (multi-L-arginyl-poly[L-aspartic acid]; Quintero et al. 2000, Valladares et al. 2002;

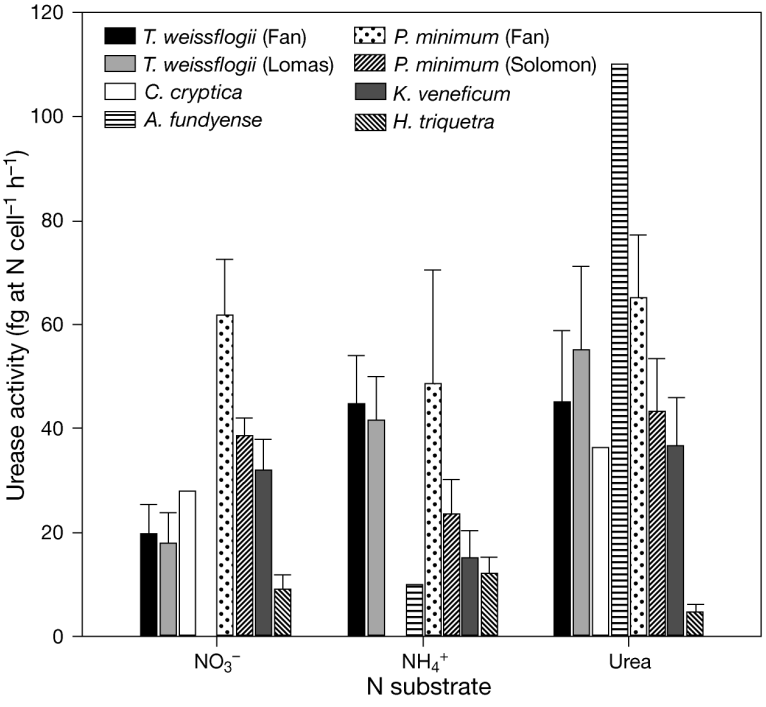

Fig. 5. Comparison of rates of urease activity in diatoms and dinoflagellates grown on different N substrates. Data for the diatoms *Thalassiosira weissflogii* were obtained from Fan et al. (2003a) and Lomas (2004), and *Cyclotella cryptica* from Oliveira & Antia (1986). Data for the dinoflagellates *Alexandrium fundyense* were obtained from Dyhrman & Anderson (2003), *Prorocentrum minimum* from Fan et al. (2003a), and *P. minimum*, *Karlodinium veneficum*, and *Heterocapsa triquetra* from Solomon & Glibert (2008). Some rates were converted from µM urea hydrolyzed min^{-1} protein^{-1} to fg-at N cell^{-1} h^{-1} using the regression from Menden-Duer & Lessard (2000) to obtain N cell^{-1} and due to the fact that from 70 to 90% of cellular N is protein (Wheeler 1983).

note that marine *Synechococcus* and *Prochlorococcus* do not have cyanophycin). This may reflect a predominant role for urease in arginine catabolism during the mobilization of N stored in cyanophycin, rather than assimilation of N from external sources of urea, in cyanophycin-containing *Cyanobacteria*.

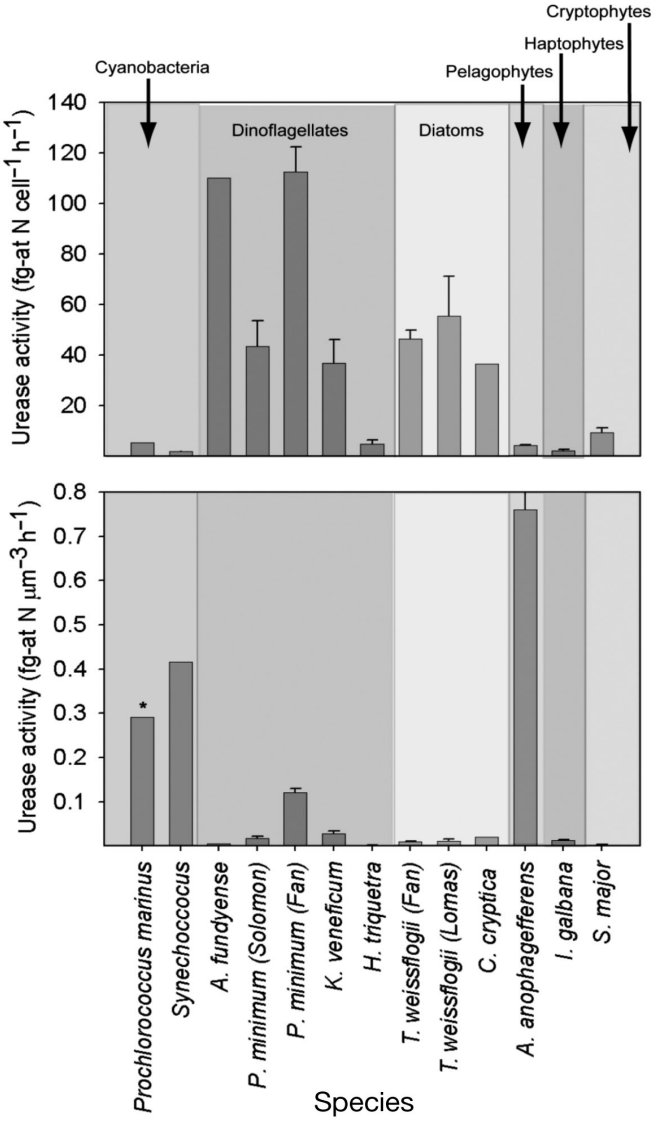

Fig. 6. Comparison of urease activity rates between different phytoplankton species grown on urea on a per cell or per cell volume basis. Data for the *Cyanobacteria Prochlorococcus marinus* and *Synechococcus* WH7805 were obtained from Palinska et al. (2000) and Collier et al. (1999), respectively. Rates were converted from µM urea hydrolyzed min^{-1} protein^{-1} to fg-at N cell^{-1} h^{-1} using 21.5 fg protein cell^{-1} for *P. marinus* (Zubkov & Tarran 2005) and 500 fg protein cell^{-1} for WH7805 (Kramer & Morris 1990). *P. marinus* on a per cell volume basis (*) was divided by 10 to allow for visualization of other species. Data sources of other species are described in Fig. 5. Cell volumes were calculated assuming a sphere or cylinder and diameters obtained from readings on the Coulter Counter or from CCMP. Full scientific names are *Alexandrium fundyense, Aureococcus anophagefferens, Cyclotella cryptica, Heterocapsa triquetra, Isochrysis galbana, Karlodinium veneficum, Prorocentrum minimum, Storeatula major* and *Thalassiosira weissflogii*

A variety of other bacteria also regulate the expression of *ure* genes in response to cellular N status via their global N regulatory systems (e.g. NTR and NAC in *Klebsiella*; AmtR in *Corynebacterium glutamicum*; TnrA, GlnR, and CodY in *Bacillus subtilis*; Mobley et al. 1995, Silberbach & Burkovski 2006, Tam et al. 2007). However, different regulatory mechanisms for the expression of urease can be found in other bacteria, including regulation by the presence of urea (via the transcription regulator UreR) in *Proteus* and *Providencia* and by pH in *Streptococcus* (Mobley et al. 1995). In some bacteria, the expression of urease is constitutive (Mobley et al. 1995).

Less is known about the regulation of UALase expression, although the theme of regulation by N availability may be consistent. *DUR1,2* is induced under N starvation or growth on 'poor' N sources (and is controlled by the transcription factor *DAL80* which negatively regulates multiple N catabolic pathways in *Saccharomyces cerevisiae*) (Cunningham & Cooper 1991). In turn, *DAL80*, a GATA repressor, is regulated by intracellular concentrations of the 'good' N sources glutamine and NH_4^+ (Cunningham & Cooper 1991, Cunningham et al. 2000, Georis et al. 2009).

Urease activity, like urea uptake, appears to have a positive relationship with temperature, in part because urease is a heat-stable enzyme (Mobley & Hausinger 1989). In the only laboratory study that investigated temperature effects, urease activity generally increased in cultures of 3 phytoplankton species over a range of temperatures (from 0 to 50°C). The study concluded that the optimal *in vitro* temperature for urease activity in *Aureococcus anophagefferens* (~50°C) was higher than that for *Thalassiosira weissflogii* and *Prorocentrum minimum* (~20°C; Fan et al. 2003a). In the field, rates of urease activity typically peak in summer months and are up to 5-fold higher than rates in fall or spring (Siuda & Chróst 2006, Solomon 2006). This seasonally recurring pattern could reflect direct or indirect effects of temperature, plankton community composition, and/or urea concentration. Higher temperatures in summer result in a greater level of heterotrophic bacterial activity and therefore increased urea production from purines than in other seasons (Berg & Jørgensen 2006). Phytoplankton taxa that tend to dominate during the summer such as dinoflagellates and *Cyanobacteria* may be utilizing this increased supply of urea. A synthesis of published rates of urease activity in culture suggests that dinoflagellates have higher urease activities on a per cell basis, while *Cyanobacteria* and one pelagophyte, *Aureococcus anophagefferens*, have higher

rates of urease activity on a per cell volume basis than other phytoplankton taxonomic groups (Solomon 2006, Glibert et al. 2008; Fig. 6). However, in field studies with diverse plankton assemblages, it has been difficult to distinguish which phytoplankton taxonomic group contributes the largest percentage of community urease activity (Solomon 2006). Urease activity in larger phytoplankton may be inhibited from a lack of Ni^{2+} (needed for the metallocenter of urease; Oliveira & Antia 1986, Egleston & Morel 2008) or by metabolites produced in the cell (urease activity measured in *in vitro* assays decreases with increasing biomass in the assay; Solomon et al. 2007). It has also been observed that most urea uptake in the field may be by eukaryotic phytoplankton, while much of the urease activity may be due to smaller phytoplankton and bacteria (Solomon 2006).

In summary, urea is primarily transported into the cell by high-affinity, active membrane transporters (encoded by the *urt* or *DUR3* genes). The rate of transport of urea into the cell is measured as urea uptake activity. Urea is also produced internally as a by-product of the urea cycle, amino acid catabolism (Mobley & Hausinger 1989, Antia et al. 1991, Siewe et al. 1998, Beckers et al. 2004), and purine catabolism (Vogels & Van der Drift 1976, McInnich et al. 2003, Allen et al. 2006). Urea produced from catabolism/salvage pathways can be either excreted into the environment (Bronk et al. 1998, Jørgensen et al. 1999b, Berg & Jørgensen 2006) or further hydrolyzed to NH_4^+ and CO_2 by the enzyme urease (encoded by the *ure* genes; Mobley & Hausinger 1989, Antia et al. 1991, Zehr & Ward 2002) or by ATP: urea amidolyase (UALase, encoded by *DUR1,2*; Antia et al. 1991, Hausinger 2004). NH_4^+ produced by catabolism of urea can be used for protein synthesis (Wheeler 1983, Capone 2000), captured by the urea cycle (Allen et al. 2006), or enter other metabolic pathways in the cell.

FUTURE DIRECTIONS IN UNDERSTANDING UREA METABOLISM AND REGULATION IN PHYTOPLANKTON AND BACTERIA

Phytoplankton genome sequences

Elucidation of the role of urea in bacterial and phytoplankton physiology is complicated by the facts that there are both external and internal sources of urea and that urea is involved in several metabolic pathways in the microbial cell (Fig. 1). The recent availability of genome sequences from several types of phytoplankton has offered a great deal of insight into the role of urea in phytoplankton metabolism. One phytoplankton taxon that plays an important part in urea utilization in marine ecosystems, but has not had a genome completely sequenced yet, is the dinoflagellates (Kudela & Cochlan 2000, Collos et al. 2004, Jauzein et al. 2008a,b). However, expressed sequence tag (EST, short mRNA sequences) libraries totaling close to 100 000 sequences from dinoflagellates, including *Karenia brevis*, *Karlodinium veneficum*, *Alexandrium tamarense*, and *Heterocapsa triquetra,* have been deposited in GenBank. BLAST comparisons have not yet revealed a gene encoding urease among the dinoflagellate ESTs, and repeated attempts to amplify fragments of the urease gene from dinoflagellates, by polymerase chain reaction, have also failed (J. L. Collier unpubl. data). BLAST comparisons have also not revealed genes for UALase among the dinoflagellate ESTs, although the fact that UALase is constructed of conserved protein domains that are also found in a variety of other enzymes, combined with the relatively short sequences provided by ESTs, make this a more difficult analysis. The EST database does contain at least 3 sequences from *Heterocapsa triquetra* (DT386240, DT385382, DT385570) and 2 from *Amphidinium carterae* (CF066550, CF065106) that appear to encode *DUR3*-like transporters. Biochemical studies testing for the presence of urease and UALase have been limited and done only on certain species of chlorophytes, prasinophytes, 1 xanthophyte (*Monodus subterraneus*), 1 chrysophyte (*Monochrysis lutheri*), and 1 diatom (*Phaeodactylum tricornutum;* Leftley & Syrett 1973, Bekheet & Syrett 1977). The standard biochemical assay for urease detects high activity in dinoflagellates (Figs. 5 & 6), but more detailed characterization of dinoflagellate urea-hydrolyzing activity would be worth pursing to discover whether the addition of co-factors for UALase (e.g. ATP) might reveal even higher activity. If dinoflagellates do not possess typical urease or UALase genes, they might have highly divergent urease or UALase, or a novel enzyme for urea degradation.

Genomes of 3 phytoplankton that are likely to have the ability to utilize urea are currently being sequenced; these genomes will add to our knowledge of the role urea plays in phytoplankton physiology. The diatom *Fragilariopsis cylindrus* and the prymnesiophyte *Phaeocystis antarctica* are common in polar seas and sea ice, where urea uptake rates vary between 0.5 and 9.4 nM-N h^{-1} (in the seas near Antarctica; Bury et al. 1995, Waldron et al. 1995, Berman & Bronk 2003). The soon-to-be-sequenced HAB diatom *Pseudo-nitzschia multiseries* releases the neurotoxin domoic acid and produces more domoic acid when grown on urea than on NO_3^- or NH_4^+ (Howard et al. 2007). Gaining an understanding of how urea metabolism influences toxin production will be instrumental in better understanding *P. multiseries* blooms.

Molecular regulation of urea metabolism genes

To better understand how urea uptake and urease activity are regulated by N availability and other environmental factors, work on regulation similar to that done on NO_3^- transporters and NO_3^- reductase (Lomas & Glibert 1999, Hildebrand & Dahlin 2000, Parker & Armbrust 2005) is needed for urea transport, urease, UALase, and urea cycle enzymes. The abundance of transcripts encoding urea transport and urease and/or UALase enzymes may be regulated by global N regulators (such as NtcA, AmtR) in bacteria in response to N availability (NO_3^-, NH_4^+, glutamine, and amino acids) in many bacteria and eukaryotic phytoplankton. Because urea transporters are located in the cell membrane, they are directly exposed to ambient NO_3^- and NH_4^+ and may be more influenced by these external N sources than by urease or UALase (Fig. 3). Despite this general expectation, transcriptional regulation would likely differ among different microbial taxa. Understanding the molecular details of how regulation of gene expression affects urea metabolism will be most useful if this understanding is integrated with biochemical measurements of rates of urea uptake and catabolism in a variety of bacteria and phytoplankton taxa.

Many of the genes involved with urea metabolism may be involved in multiple pathways that must be taken into consideration when studying their regulation. For example, urease can be involved in both recycling 'old' N (from amino acid or purine catabolism) within a cell and making 'new' N available from purines, amino acids, or urea taken up from outside the cell. In addition to making urea available as a source of N, urease may play other roles in some bacteria. For example, NH_3-oxidizing bacteria may use the NH_3 released from urea by urease as a source of energy, while other bacteria (including some pathogens) use the net increase in pH caused by hydrolysis of urea as a mechanism for surviving acidic conditions (Mobley et al. 1995, Koper et al. 2004). Likewise, the enzymes in the urea cycle are involved in pathways that produce intermediates that are possibly important in other aspects of cellular metabolism (Armbrust et al. 2004, Allen et al. 2006).

Importance of studying urea metabolism

A better understanding of the metabolism of urea is needed to support investigations of important issues in plankton ecology. For example, the supply of urea from anthropogenic sources has been linked to increased occurrences of HABs (e.g. Berg et al. 1997, Glibert et al. 2006, Sanderson et al. 2008). Learning exactly how HAB species are able to take advantage of the urea-N supply to outgrow their competitors is essential in managing HABs. The timing of urea inputs into these vulnerable ecosystems may be important in determining the effect of anthropogenic urea enrichment because of differences in the potential to use urea among the species present and how that potential is influenced by environmental factors such as N availability, temperature, and irradiance. In plankton communities that are not heavily affected by anthropogenic urea, similar information will be helpful in understanding which organisms are responsible for urea uptake and urea decomposition under various conditions. As worldwide use of urea as a N fertilizer and feed additive has increased more than 100-fold in the past 4 decades, and doubled in just the past decade (Glibert et al. 2006), the effects of anthropogenic urea are expected to increase into the future, and questions regarding the physiological and biochemical regulation of this nutrient by microbes will be increasingly important to resolve.

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