Microbes and the Marine Phosphorus Cycle

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Phosphorus (P) is fundamental to life, and years of study in marine systems have built a broad understanding of the marine P cycle. Various aspects of marine P biogeochemistry have been reviewed previously (Benitez-Nelson, 2000; Paytan and McLaughlin, 2007). Here, we focus on recent advances in our understanding of marine P and the interactions between microbes and the P cycle. These advances come from a variety of disciplines, but generally highlight three main themes: (1) ocean microbes are adapted for surviving in a variable P environment, (2) the dissolved organic phosphorus (DOP) pool likely plays a critical role in driving growth, metabolism, and community composition of ocean microorganisms, and (3) P is very rapidly cycled, which highlights its importance in marine systems.

THEME 1.
ADAPTATION TO VARIABLE P

Organisms have evolved mechanisms, or adaptations, for dealing with changing P in the marine environment. Many of these adaptations have not been examined until recently, and they highlight that P, just like nitrogen (N), iron (Fe), or light may drive microbial evolution and niche adaptation. Recent advances give researchers a better understanding of P conservation, P limitation responses, and P niche partitioning, which are three primary adaptations to variable P in the ocean.

Planktonic microbes in low-P environments (e.g., the Sargasso Sea) are particularly frugal with P that they acquire from their environments. Phospholipids and nucleic acids appear to be the primary reservoirs of P within the planktonic cells in the open ocean (Van Mooy et al., 2006; recent work of author Van Mooy and Allan Devol, University of Washington, paper in review), and recent studies show that plankton have evolved mechanisms to economize on these biochemical P requirements. For example, using genomic evidence and direct observations, Van Mooy et al. (2006) show that *Prochlorococcus* and *Synechococcus*, the picocyanobacteria that often dominate low-P environments, primarily synthesize a type of membrane lipid that contains sulfur and sugar rather than more common lipid forms that contain phosphate (e.g., phospholipids). This switch from P-lipids to S-lipids decreases cellular demand for P and may be an important adaptation of picocyanobacteria to the low-P environments in which they frequently thrive.

The synthesis of genomic DNA may compose as much as half of the total P demands of picocyanobacteria (Bertilsson et al., 2003). Thus, it would behoove these plankton to retain the absolute minimum amount to of genetic information. Indeed, *Prochlorococcus* possess the smallest genome of any photosynthetic organism. Heterotrophic bacteria also appear to have adapted this strategy; *Pelagibacter ubique*, the ubiquitous heterotrophic marine bacterium that dominates the open ocean, maintains the smallest number of predicted open reading frames, or genes, of any free-living organism (Giovannoni et al., 2005). Although other marine bacterial genomes are much larger and have more extensive and numerous global regulatory systems, *P. ubique* has maintained only four two-component regulatory systems and one of these is dedicated to responses associated with P limitation (PhoR/PhoB/PhoC) (Giovannoni et al.,

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2005). This highlights that this organism has dedicated a relatively significant fraction of its overall P demand to maintain an ability to deal with variations in P supply and P limitation in the ocean.

An additional genome-enabled observation about P limitation responses is the frequent presence of genes related to the high-affinity uptake of phosphate in the Sargasso Sea environmental genome (SSEG) sequencing project (Venter et al., 2004) and the genomes of marine cyanobacteria, viruses, and eukaryotic phytoplankton. For example, there are numerous copies of the gene for the high-affinity, phosphate-binding protein PstS in the SSEG (Venter et al., 2004). There are also multiple copies of PstS in the marine cyanobacterial genomes sequenced to date, including different *Prochlorococcus* ecotypes (Moore et al., 2005) and the nitrogen-fixing genera *Trichodesmium* (Orchard et al., 2003) and *Crocosphaera* (Dyhrman and Haley, 2006). Two cyanophage myoviruses (P-SSM2 and P-SSM4) that infect *Prochlorococcus* also both have copies of PstS (Sullivan et al., 2005). This indicates the potential importance of P to cyanophages and their hosts, with PstS expression possibly serving to enhance P acquisition during the infection of P-limited hosts (Sullivan et al., 2005) (Figure 1, Area 1). P-related genes are not exclusive to the cyanophages. In fact, a putative phosphate-repressible permease (ehv117) is present in the genome of the coccolithovirus EhV-86 (Wilson et al., 2005). The coccolithophore host for this virus, *Emiliania huxleyi*, also has a putative phosphate-repressible permease (Corstjens et al., 2003), which is up-regulated under low-P conditions (Dyhrman et al., 2006b). Taken together, these observations highlight that marine microbes are adapted for surviving in a variable P environment.

THEME 2. IMPORTANCE OF DISSOLVED ORGANIC PHOSPHORUS

While P*i* is generally regarded as the most bioavailable form of P, its concentration in the surface waters of the open ocean is much smaller than that of DOP (Figure 1). Yet, we know surprisingly little about the chemical composition or bioavailability of DOP (for a thorough and relatively recent review of these issues, see Karl and Björkman, 2002). Not unlike DOC and DON, only a minor fraction of the chemicals that compose DOP are amenable to direct molecular characterization. For example, dissolved nucleotide triphosphates, which are involved in cellular energy storage and numerous biosynthetic pathways, can be directly isolated and quantified. However, they were found to compose less than one percent of total DOP in the North Pacific subtropical gyre (Björkman and Karl, 2005). Operational definitions for the molecular composition of DOP, such as hydrophobicity or susceptibility to enzymatic attack (e.g., Suzumura and Ingall, 2004; Suzumura et al., 1998), can generally define larger fractions of DOP, but this type of information is difficult to apply to the assessment of the sources and fates of DOP.

As an alternative to direct molecular analyses, solid-state ^{31}P-NMR analysis provides a critical view of the chemical bonding environment of P atoms in the high molecular weight (HMW) DOP, which comprises about a quarter of total DOP in the upper ocean (Clark et al., 1998; Kolowith et al., 2001). This approach shows that the majority of HMWDOP is contained in phosphoester (C-O-P) bonds that are characteristic of biochemicals. However, nucleic acids and lipids contain P atoms in phosphodiester bonds almost exclusively, and solid-state ^{31}P-NMR is not able to distinguish phosphomonoester or phosphodiester bonds. Thus, it is difficult to assess to what extent HMWDOP is composed of intact and potentially bioavailable P-containing biochemicals or their potentially refractory degradation products (Figure 1, Area 2). Evidence for the latter scenario also comes from ^{31}P-NMR, which shows that the remaining quarter of DOP not contained in phosphoester bonds is contained in phosphonate (C-P) bonds (Figure 1, Area 3) that are almost entirely absent in planktonic biomass. This suggests that HMWDOP may represent some minor component of DOP production that is not suscep-

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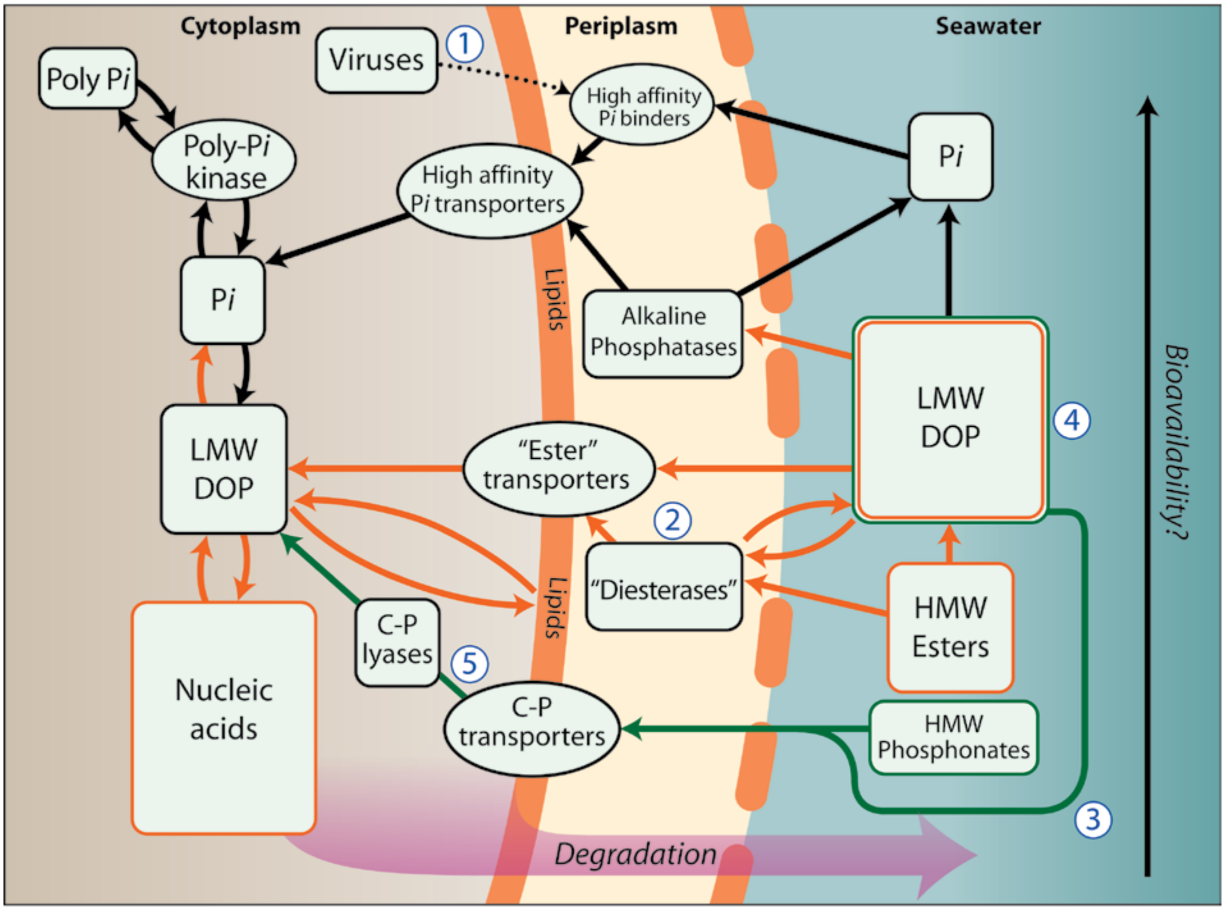

Figure 1. A conceptual model of dissolved P pools, their bioavailability, and P transformations across the prokaryotic cell membrane. The phosphate pool and pathway is indicated in black, phosphoesters in orange, and phosphonates in green. Note the relative size of the different P pools; their likely bioavailability is indicated on the right of the diagram. In this conceptual model, we indicate the potential for microbial metabolism of phosphate (through a high affinity system), general phosphoesters (via hydrolysis by nucleases or phospholipases, for example), phosphomonoesters (via hydrolysis by alkaline phosphatase – AP), and phosphonates (via a C-P lyase). We underscore that these are conceptual routes, and the presence and the localization of the transporters and enzymes shown here may differ substantially between microbes. For example, the route of phosphonate transport, hydrolysis, and accumulation as either P*i* or LMW DOP is not well characterized. We also highlight here some important areas of P biogeochemistry that are poorly understood and deserve further attention: (1) the presence and functional role of viral P-related genes; (2) the reactivity of HMW phosphoesters, their modes of hydrolysis, and their transport into the cell; (3) the sources and cycling of dissolved phosphonates; (4) the composition and bioavailability of LMWDOP; and (5) the frequency and specificity of microbial phosphonate metabolism.

tible to enzymatic attack and has thus accumulated in the sea. The observation that phosphonate and phosphoester HMWDOP occur in fixed ratios in the mixed layer and the mesopelagic layer also supports this argument. However, HMWDOP is more concentrated in the mixed layer than in the mesopelagic layer, and thus a significant fraction of HMWDOP, including phosphonates, must be bioavailable on time scales of upper-ocean mixing (months to years). Yet, while ^{31}P-NMR has given us important constraints on the P bond classes in particulate matter and HMWDOM (e.g., polyphosphates are rare in both), these classes are likely to be composed of a broad diversity of P-containing organic molecules possessing a range of bioavailabilities. For example, phosphoester compounds dominate particulate phosphorus (PP) in the surface ocean and HMWDOP in the deep ocean, yet these reservoirs of organic matter turn over on time scales of days and centuries, respectively. Finally, low molecular weight (LMW) DOP is not yet amenable to NMR analysis, and, consequently, there currently is almost no information available on the majority of the DOP pool (Figure 1, Area 4). However, studies of the elemental stoichiometry of DOM and sinking organic matter suggest that organic P, in general, is more reactive than organic N or C (Benitez-Nelson and Karl, 2002; Church et al., 2002; Karl et al., 2001b; Sannigrahi et al., 2005).

Although there remains a woefully inadequate understanding of the linkages between organic P composition and bioavailability, recent molecular-biological studies show that the relative bioavailability of P, whether present as P*i*, DOP, or PP, is a potentially important driver of microbial niche partitioning in the sea. For example, there appear to be substantial differences in the presence, topology, and regulation of genes thought to be involved in P acquisition between strains of *Prochlorococcus* (Martiny et al., 2006), which may be a function of their adaptation to different P regimes. Another example of niche partitioning is our recent understanding of the diversity of strategies for the utilization of P from DOP, such as from phosphomonoesters or phosphonates (Figure 1). In the picocyanobacteria, such as *Prochlorococcus*, some strains appear to be able to hydrolyze phosphomonoesters, whereas others do not (Martiny et al., 2006). Furthermore, even when microbes may have the ability to hydrolyze phosphomonoesters, this activity may be regulated differently between different genera, and even different strains of the same species over small spatial scales (Dyhrman et al., 2002). With the resolution to identify alkaline phosphatase activity in single cells, it appears that there may be considerable heterogeneity in the niche partitioning of planktonic microbes with regard to phosphomonoester hydrolysis. This may also be the case for phosphonate hydrolysis. A combination of genomic, culture, and field observations suggests that *Trichodesmium* has the genetic capacity to metabolize phosphonate compounds (Figure 1, Area 5). Phosphonates were generally considered to be an unavailable form of P for phytoplankton growth until the release of the marine cyanobacterial genomes revealed genes putatively involved in phosphonate metabolism (Dyhrman et al., 2006a; Martiny et al., 2006; Moore et al., 2005). The ability to metabolize phosphonate compounds may explain why *Trichodesmium* is so successful in low-phosphate environments. Although *Synechococcus* may be able to use certain phosphonate compounds (Palenik et al., 2003), the strains and phosphonate compounds tested to date suggest that other picocyanobacteria (*Crocosphaera* and *Prochlorococcus*) are unable to metabolize exogenous phosphonate as a sole P source (Dyhrman and Haley, 2006; Martiny et al., 2006; Moore et al., 2005). As such, *Trichodesmium* appears to occupy a unique niche with regard to P metabolism among marine cyanobacteria.

In summary, the DOP pool appears to play a critical role in driving growth, metabolism, and community composition of ocean microorganisms. However, there are many remaining gaps in our understanding of DOP cycling, particularly the composition of the LMWDOP and the sources of phosphonate compounds to the HMWDOP.

THEME 3. RAPID P CYCLING

P is rapidly cycled by planktonic microbes and is increasingly viewed as an important deficient, or even limiting, nutrient in some regions of the open ocean and in some estuarine and river-dominated coastal systems. Low-nutrient, low-chlorophyll (LNLC) regions, including many oligotrophic central gyres, are dominated by prokaryotic plankton, both autotrophs and heterotrophs. Regions such as the Sargasso Sea in the northwestern Atlantic (Fanning, 1992), the Mediterranean Sea (especially the eastern part [Krom et al., 1991]), and the North Pacific subtropical gyre (Karl et al., 2001b) all have low P with evidence for planktonic P stress either in the heterotrophic bacteria or the phytoplankton. There is also growing seasonal evidence of P stress in coastal systems dominated by high-N freshwater inputs. Below, we highlight some new observations from these different systems.

P concentrations at the Bermuda Atlantic Time Series (BATS) station are 10 nM or less (Wu et al., 2000) and are even lower to the south (Cavender-Bares et al., 2001). Half-saturation (K_m) values for P*i* uptake (~10 nM) reflect these low P concentrations and P*i* turnover times are 12 hours or less (Ammerman et al., 2003). Several studies from different laboratories have described P limitation of heterotrophic bacterial growth or respiration in the Sargasso Sea (e.g., Cotner et al., 1997), or N + P limitation, though others have also stressed the importance of carbon limitation (Carlson and Ducklow, 1996; Carlson et al., 2002). Seasonal differences and the influence of eddies are likely to account for some of this variation (Carlson et al., 2004). Phytoplankton P stress at BATS appears to be widespread, but varies among taxonomic groups (Dyhrman et al., 2002, Lomas et al., 2004). Additional studies in other regions of the Atlantic Ocean also highlight the importance of P bioavailability, where investigators in the tropical and eastern Atlantic have demonstrated P limitation (Sañudo-Wilhelmy et al., 2001) or both P and Fe limitation (Mills et al., 2004) of N fixation.

P is generally considered to be the major limiting nutrient for both phytoplankton and bacteria in much of the Mediterranean, though N and Fe are also important (Thingstad et al., 1998). P concentrations in the eastern Mediterranean are even lower than in the Sargasso Sea, with concentrations less than 2 nM and P*i* turnover times of two to four hours (Thingstad et al., 2005). The eastern Mediterranean was also the site of the recent CYCLOPS (Cycling of Phosphorus in the Mediterranean) experiment, the only large-scale marine P addition conducted to date (Krom et al., 2005; Thingstad et al., 2005). This P addition had unexpected food web effects, with most of the added P bypassing the phytoplankton and cycling through the heterotrophic bacteria to higher trophic levels (Thingstad et al., 2005). Numerous studies have also been conducted in the northwestern coastal Mediterranean (Pinhassi et al., 2006), with clear evidence for bacterial P limitation and P*i* turnover times of two hours or less (Thingstad et al., 1998). A recent study shows that the bacterial community composition was influenced by the seasonal pattern of nutrient limitation in the region, with some bacterial groups absent during the period of lowest P concentrations in spring and summer (Pinhassi et al., 2006). P stress has also been studied in the Gulf of Aqaba, in the northern Red Sea not far from the Mediterranean, where seasonal variations in PstS gene expression (a common indicator of microbial P limitation) was highest in the summer, the period of lowest P concentration (Fuller et al., 2005).

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The Hawaii Ocean Time-series (HOT) has extensively documented a "domain shift" in the North Pacific subtropical gyre (Karl et al., 2001a) since the 1980s or before. Dissolved P and silicate concentrations have declined, phytoplankton chlorophyll a and primary production have doubled, and the plankton populations have become increasingly dominated by prokaryotes, apparently in response to climatic changes (Karl et al., 2001a, 2001b). The role of P has become more important because of these changes (Karl et al., 2001b), though P concentrations are higher (~ 40 nM) and P*i* turnover times slower (median nine days) (Björkman et al., 2000) than in the Sargasso Sea or Mediterranean.

Although most pristine estuarine and coastal ecosystems are light-, N-, or Fe-limited, seasonal P limitation is becoming more common in estuaries and river-dominated continental shelf waters as global N loading from agriculture and other sources increases (Howarth and Marino, 2006). This P limitation occurs during the high-discharge spring period and may be followed by summer N limitation, as recently demonstrated on the Louisiana shelf (Sylvan et al., 2006). In this system, P*i* turnover times were less than 30 minutes during the strongest P limitation in May and July (Sylvan et al., 2006), and there is also evidence for P limitation of heterotrophic bacteria in this region (Chin-Leo and Benner, 1992). Similar seasonal patterns of P limitation have also been observed in the Neuse River (Paerl et al., 2004), Chesapeake Bay (Fisher et al., 1999), Delaware Bay (Pennock and Sharp, 1994), some subestuaries of the Baltic Sea (Conley, 1999), and the Danube River plume and nearby shelf waters of the Black Sea (Ragueneau et al., 2002). Coastal waters near Hong Kong are also P limited in the summer because of nutrient input from the Pearl River (Miao et al., 2006). Many of these systems are also suffering from the effects of eutrophication, including summer hypoxia (Diaz, 2001) and harmful algal blooms (Glibert et al., 2005). Continuing increases in N loading worldwide are likely to further increase the potential for P limitation in estuarine and coastal ecosystems.

To conclude, process studies and a recent suite of genome-enabled investigations are expanding our understanding of adaptation to P in marine microorganisms. These adaptations range from P conservation to P niche partitioning. In fact, the highly specialized adaptations of modern marine microbes to rapid cycling P*i* suggests that intense competition for P between phytoplankton and heterotrophic bacteria has been a major force in the evolution of marine microbes, and once again underscores that P is an important driver of microbial dynamics in marine systems. Evidence of P limitation and P competition continues to mount in a variety of open-ocean and coastal systems. Despite these many advances in our understanding of the P cycle and appreciation for its significance, many uncertainties about microbial P metabolism and P cycling (e.g., the constituents of the LMWDOP) remain, and studies on marine microbes and the P cycle will continue to be a critical area of future research.

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