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Raphaël Lami[1,2,3,*], David L. Kirchman[1]

[1]University of Delaware, School of Marine Science and Policy, Lewes, Delaware 19958, USA
[2]Sorbonne Universités, UPMC Univ Paris 06, USR 3579, LBBM, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France
[3]CNRS, USR 3579, LBBM, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France

ABSTRACT: Proteorhodopsins (PR) are phylogenetically widespread and highly expressed proton pumps in marine bacterial communities, including in the SAR11 clade, one of the most common clades in the oceans. The relationships between PR expression, light and cell activity remain unclear, especially in natural environments. We examined these relationships during 3 diurnal studies in spring, summer and fall in Delaware coastal waters. The abundance of genes and transcripts of SAR11 PR, SAR11 16S rRNA and total bacterial 16S rRNA were monitored using a quantitative PCR approach. We found that the expression of SAR11 PR was 2.5-fold higher during the day than at night. However, SAR11 16S rRNA levels remained constant during the day and night on all cruises, suggesting that the growth-related activity of SAR11 was not directly affected by sunlight. There was a tight correlation between expression of PR in SAR11 and photosynthetically active radiation, but not with other environmental parameters. Our data support the hypothesis that light affects PR expression by SAR11 populations, but the energy from PR appears to contribute relatively little to supporting bacterial growth-related activity in marine waters.

KEY WORDS: Marine bacteria · SAR11 · Proteorhodopsin · Quantitative PCR

INTRODUCTION

Proteorhodopsins (PR) constitute a major group of proteins that are expressed in the marine environment. Since their discovery in 2000 (Béjà et al. 2000), several studies have revealed their broad phylogenetic and geographic distribution (de la Torre et al. 2003, Finkel et al. 2013) and their high abundance in marine waters (Venter et al. 2004, Rusch et al. 2007, Campbell et al. 2008, Finkel et al. 2013). These data suggest the importance of this protein in bacterial physiology and for potentially regulating energy fluxes in the oceans.

The physiological roles of PR are currently being investigated. Although some rhodopsins in marine microbes may be light sensors (Fuhrman et al. 2008), experimental studies and sequence analyses have indicated that PR are proton pumps, enabling photophosphorylation and cell motility (Walter et al. 2007). Recent studies have found that PR promotes the survival of marine bacteria during starvation (Gómez Consarnau et al. 2010, Steindler et al. 2011). Also, Wang et al. (2012) showed that PR can be expressed in vibrios under respiratory stress, while Feng et al. (2013) found higher PR expression in the flavobacterium *Psychroflexus torquis* when grown in salinities below or above optimal levels. Results from these previous studies are consistent with the hypothesis that light provides additional energy for PR-containing bacteria starved of organic carbon or stressed by low nutrient concentrations or other environmental properties.

However, the effects of light on the growth-related activity of PR-containing bacteria remain unclear.

*Corresponding author: raphael.lami@obs-banyuls.fr

Light does not affect the growth of a cultivated representative of the SAR11 clade (Giovannoni et al. 2005, Steindler et al. 2011), and the gammaproteobacterium SAR92 HTCC2207 and the flavobacterium *Dokdonia* PRO95 grew equally well under dark and light (Stingl et al. 2007, Riedel et al. 2010). In contrast, *Dokdonia* MED134 has been shown to grow faster with light (Gómez-Consarnau et al. 2007, Kimura et al. 2011), and salinity-stressed *P. torquis* also grows faster with light, although there was no effect when it was grown under organic carbon limitation (Feng et al. 2013). In one of the few studies with natural communities, light did affect the growth of SAR11 bacteria in microcosms exposed to artificial light–dark cycles (Lami et al. 2009a). Also, the fraction of cells using leucine in the light was 25% higher for SAR11 bacteria than for the total bacterial community in Delaware Bay, although the effect was not consistently seen (Straza & Kirchman 2011). Other studies have seen either no effect or even inhibition by light of the activity of natural microbial communities with potential PR-containing bacteria (Michelou et al. 2007, Straza & Kirchman 2011, Ruiz-González et al. 2012a,b).

A few contradictory studies have examined the effects of light on PR expression. Light significantly induces PR expression in *Dokdonia* MED134 cells grown in pure culture (Gómez-Consarnau et al. 2007, Kimura et al. 2011), while Steindler et al. (2011) found PR expression by a *Candidatus* Pelagibacter strain (HTCC1062) to be 2-fold higher in the dark compared with the light, although mass spectrometry data indicate that the PR protein is present in the light and dark (Giovannoni et al. 2005). In contrast, PR transcript levels were similar in light and dark for *Dokdonia* PRO95 (Riedel et al. 2010). Interestingly, Akram et al. (2013) found that expression of PR was higher at the transition from the exponential growth phase to the stationary phase for *Vibrio* sp. AND4. These authors argued that nutrients, not light exposure, regulated PR expression by this bacterium. During a microcosm experiment with natural microbial communities, light-dependent PR expression was observed in SAR11 and flavobacterial groups (Lami et al. 2009a), and PR transcripts were more abundant during the day than at night in the North Pacific Ocean (Poretsky et al. 2009), but not in coastal waters of northern California (Ottesen et al. 2013). These data indicate the need to better characterize the relationships between light, other environmental conditions and PR expression. In particular, it is not clear whether light stimulates PR expression by natural marine communities and whether this expression is tied to enhanced growth-related activity by PR-containing bacteria.

In this study, we examined the expression of PR and 16S rRNA by bacteria in the SAR11 clade under natural day–night cycles during 3 cruises in Delaware coastal waters. SAR11 bacteria are often the most abundant taxa in marine systems (Morris et al. 2002), including Delaware coastal waters (Campbell et al. 2011). We used a quantitative PCR (qPCR) approach to examine the genes and transcripts of PR and 16S rRNA for SAR11 and the total bacterial community. The total diversity of 16S rRNA genes was also determined by 454 pyrosequencing to explore overall changes in the bacterial communities during the 3 diurnal studies. We observed light-dependent relative expression of SAR11 PR during all cruises, but light did not seem to directly affect activity of SAR11 bacteria.

MATERIALS AND METHODS

Water samples were collected aboard the R/V 'Hugh R. Sharp' from Delaware coastal waters (38° 50.935' N, 75° 6.456' W) at 1 m depth using a rosette of Niskin bottles mounted on a CTD profiler. The light sensor was a Biospherical PNF-210 radiometer. The cruise dates were 3–4 May 2007, 20–21 September 2007 and 8–9 September 2008. Samples were taken about every 4 h. All samples were processed immediately onboard. A total of 1 l was filtered for each sample, under reduced light intensities overnight to minimize the effects of filtration on gene expression. All data are expressed as a function of solar time, which was 2 h before local time during these cruises.

Environmental parameters and nucleic acid extraction

Total prokaryote abundance, bacterial production and chlorophyll *a* (chl *a*) were measured following previously described protocols (Cottrell et al. 2006). For pyrosequencing and qPCR analyses, all water samples were pre-filtered through 0.8 µm polycarbonate filters to minimize the presence of eukaryotic DNA, and the <0.8 µm size fraction was collected on 0.22 µm Durapore filters. Filters were then placed in 2% N-cetyl N,N,N-trimethylammonium bromide and stored onboard at 80°C. Total DNA was extracted using standard protocols (Dempster et al. 1999) and quantified via a standard picogreen assay (Invitrogen) on a POLARstar Optima fluorometer (BMG

Labtech). Total RNA was co-extracted with DNA and isolated using the following mix (Turbo DNA-free Kit, Ambion): 0.5 units of DNase I per 100 ng of total nucleic acids with 1 µl of 10× buffer (Ambion) (30 min, twice, 37°C). Total RNA was quantified via a ribogreen assay (Invitrogen) on the POLARstar Optima fluorometer. DNA removal from RNA samples was confirmed by PCR amplification without the reverse transcription step, following the PCR conditions described below. No amplification was detected in these controls.

Quantification of PR gene and transcript abundance

SAR11 PR genes and transcripts were targeted by qPCR. The SAR11 PR primers and PCR conditions were the same as those described in Lami et al. (2009a). One PR-containing plasmid was used as a positive control to establish standard curves in the qPCR assays. This PR gene, similar to the one in the targeted SAR11 populations (data not shown), was isolated from environmental clones (Qiaprep Kit, Qiagen) and linearized with PstI (Invitrogen). Standard reactions with linearized plasmid contained approximately 10^1 to 10^6 copies per reaction. All standard curves were linear within the ranges tested.

Quantitative PCR was performed in triplicate or quadruplicate with 1 µl of diluted DNA (0.1 ng µl^{-1}) or 1 µl of diluted RNA (0.05 ng µl^{-1}) in a final volume of 12.5 µl using the Stratagene SYBR green mix with an ABI 7500 (Applied Biosystems). The PCR conditions were an initial denaturation step at 95°C (10 min for qPCR, 5 min for real-time qPCR), followed by 30 to 40 cycles of amplification at 95°C for 15 s, the indicated annealing temperature for 45 s, and 72°C for 45 s, with a final dissociation step. No reverse transcription was performed during the qPCR reactions. For real-time qPCR, an additional step was added before the denaturation step (40°C, 1 min). Final primer concentrations were 0.2 µM. Only single peaks were observed in the dissociation curves for both the standards and samples, indicating specific amplification with each set of primers. Amplification efficiencies were between 92 and 103%, and no inhibition was detected when a known quantity of standard was added to each sample (data not shown). The detection limit of qPCR assays was about 50 copies of genes or transcripts per ml. The specificity of the SAR11 PR primers has been previously confirmed, as discussed in more detail by Campbell et al. (2008).

Sequence analysis of 16S rRNA genes

All 16S rRNA genes were pyrosequenced on a Roche 454 FLX instrument (Research and Testing Laboratory) using a mixture of Hot Start and HotStar high-fidelity *Taq* polymerases, which generated amplicons of 250 to 550 bp starting from position 27 (*Escherichia coli* 16S rRNA number). Sequences were run through AmpliconNoise and Perseus (Quince et al. 2009) to remove noise and chimeras from the original SFF files. The 16S rRNA sequences were de-multiplexed and quality filtered, and homopolymers were removed using QIIME (Caporaso et al. 2010). QIIME was also used to calculate alpha- and beta-diversity indices (Caporaso et al. 2010). Sequences are available at the European Nucleotide Archive, accession number ERP003436/PRJEB4174.

RESULTS

Environmental parameters and microbial diversity

Salinity was relatively constant during the 3 cruises, varying between 30.4 and 31.6 PSU (Table 1). Chl *a* concentrations were low in May 2007 (1.61 ± 0.03 [SD] to 2.94 ± 0.04 µg l^{-1}) and higher during September 2007 and 2008 (8.4 ± 0.1 to 15.6 ± 0.7 µg l^{-1}). Bacterial abundances varied overall between $1.56 \times 10^6 \pm 5.68 \times 10^5$ and $6.9 \times 10^6 \pm 0.2 \times 10^6$ cells ml^{-1} (Table 1). Bacterial production (leucine incorporation) was lower in May 2007, varying from 16 to 54 pM h^{-1}, than in September 2007, when it varied between 86 and 151 pM h^{-1} (Fig. 1). Production was highest in September 2008 (between 116 and 397 pM h^{-1}).

Light penetration in the water column, as indicated by the attenuation coefficient, varied from 0.66 to 0.21 m^{-1} during May 2007, from 0.6 to 0.54 m^{-1} during September 2007, and from 0.73 to 0.63 m^{-1} during September 2008. Photosynthetically active radiation (PAR) was between 160 and 2050 µmol m^{-2} s^{-1} in May and between 110 and 2990 µmol m^{-2} s^{-1} during the September cruises (Table 1).

UPGMA clustering revealed 3 groups of 16S rRNA gene pyrosequences (97% similarity) representing the entire bacterial communities sampled by the 3 cruises. 16S rRNA genes varied more between the cruises than during one cruise (Fig. 2). Similarly, there was no significant correlation between 16S rRNA diversity and the tides (data not shown).

The SAR11 clade dominated the community composition during all cruises, making up 32 to 37% of the total sequences (Table 2). The SAR86 clade was

Table 1. Selected biogeochemical parameters during the 3 cruises in Delaware coastal waters. PAR = photosynthetically active radiation. Inc. = incorporation, Abund. = Abundance, Atten. = Attenuation

Cruise Solar time (h)	Temp. (°C)	Salinity (PSU)	PAR (µmol m^{-2} s^{-1})	Atten. (m^{-1})	Chl *a* (µg l^{-1})	SD	Abund. (10^6 cells ml^{-1})	SD	Leucine inc. (pM h^{-1})	SD
May 2007										
09:00	11.7	31.2	1460	0.66	2.92	0.04	1.9	0.5	20.3	2.3
13:00	12.1	31.3	1870	0.42	2.43	0.09	1.6	0.5	33.6	7.3
17:00	12.2	31.4	160	0.39	1.62	0.03	2.0	0.5	16.0	9.5
21:00	12.3	31.0	Night	Night	1.69	0.05	2.5	0.6	53.7	3.6
01:00	11.8	31.4	Night	Night	2.07	0.10	3.9	0.5	37.1	2.9
05:00	11.8	31.3	240	0.43	2.31	0.05	3.0	0.1	31.1	5.1
09:00	11.8	31.5	2050	0.21	1.95	0.05	2.3	0.4	31.1	2.2
Sep 2007										
06:30	21.3	31.4	110	Night	8.41	0.14	3.7	0.7	150.7	7.6
10:30	21.2	31.5	224	0.6	9.63	0.10	4.2	0.5	97.9	4.7
14:30	21.8	31.5	1900	0.59	8.23	0.92	3.7	0.7	128.0	11.9
18:30	21.6	31.6	Night	Night	7.25	0.33	4.0	0.4	106.2	17.6
22:30	21.2	31.5	Night	Night	8.48	0.32	3.3	0.3	96.0	1.0
02:30	21.2	31.5	Night	Night	8.52	0.24	3.3	0.6	85.8	7.5
06:30	21.3	31.5	2990	0.56	9.50	0.28	2.9	0.8	94.6	7.9
10:30	21.3	31.5	2210	0.54	10.22	0.25	3.6	0.7	91.1	11.7
Sep 2008										
07:00	22.8	30.7	1727	0.7	13.18	0.73	6.3	0.8	191.4	14.2
11:00	23.2	30.8	2160	0.69	13.73	0.64	4.4	0.8	115.6	4.3
15:00	23.3	30.8	1360	0.64	14.02	0.18	1.2	0.2	235.0	16.2
19:00	23.5	30.8	Night	Night	12.48	0.55	3.9	0.1	289.9	19.0
23:00	23.2	30.7	Night	Night	13.13	0.16	4.7	0.7	249.3	7.2
03:00	23.1	30.7	Night	Night	12.43	0.06	5.7	0.2	396.5	3.6
07:00	23.1	30.6	630	0.73	14.48	0.50	6.9	0.2	240.5	12.4
11:00	23.8	30.4	1460	0.66	14.80	0.48	6.2	0.2	303.6	11.7
15:00	23.4	30.4	270	0.63	15.60	0.65	6.5	0.1	199.4	12.5

Table 2. Relative abundance of major bacterial clades examined in this study during the 3 cruises in Delaware coastal waters, given as % of total sequences. Sampling between 19:00 and 02:30 h was at night (table continues on next page)

May 2007 cruise	Day 1 09:00	Day 1 13:00	Day 1 21:00	Day 2 01:00	Day 2 09:00	Average	SD
SAR11	34.6	35.6	36.8	41.3	39.2	37.1	3.0
Other Alphaproteobacteria	14.0	13.4	15.5	13.1	15.3	14.0	1.1
SAR86	12.3	10.7	11.7	10.8	10.6	11.4	0.8
Other Gammaproteobacteria	3.5	4.2	3.6	3.1	2.5	3.6	0.5
Bacteroidetes	9.4	10.1	9.4	8.3	12.3	9.3	0.7
Cyanobacteria	0.7	0.9	1.0	0.6	6.9	0.8	0.2
Actinobacteria	7.9	6.4	6.9	7.9	1.2	7.3	0.8
Other groups	17.6	18.7	15.1	14.9	12.0	16.6	1.9
Sep 2007 cruise	Day 1 10:30	Day 1 14:30	Day 1 22:30	Day 2 02:30		Average	SD
SAR11	37.2	32.9	38.2	29.0		34.3	4.2
Other Alphaproteobacteria	11.7	14.6	14.6	12.7		13.4	1.4
SAR86	7.5	7.3	8.6	11.5		8.7	1.9
Other Gammaproteobacteria	4.2	4.2	4.3	4.4		4.3	0.1
Bacteroidetes	6.8	11.4	9.2	9.9		9.3	1.9
Cyanobacteria	13.8	13.2	7.6	12.2		11.7	2.8
Actinobacteria	9.6	7.7	8.0	8.5		8.5	0.8
Other groups	9.2	8.7	9.5	11.8		9.8	1.4

Table 2 (continued)

Sep 2008 cruise	Day 1 07:00	Day 1 11:00	Day 1 19:00	Day 1 23:00	Day 2 07:00	Average	SD
SAR11	33.8	31.8	23.5	37.4	27.2	31.6	5.9
Other Alphaproteobacteria	16.2	16.6	17.2	16.4	13.9	16.6	0.4
SAR86	11.0	13.0	12.1	8.8	8.3	11.2	1.8
Other Gammaproteobacteria	2.6	2.8	2.5	1.7	5.2	2.4	0.5
Bacteroidetes	14.9	14.4	14.9	18.3	10.4	15.6	1.8
Cyanobacteria	6.8	8.7	12.1	5.7	13.0	8.3	2.8
Actinobacteria	2.1	1.4	2.2	1.5	8.6	1.8	0.4
Other groups	12.6	11.3	15.5	10.2	13.4	12.6	2.3

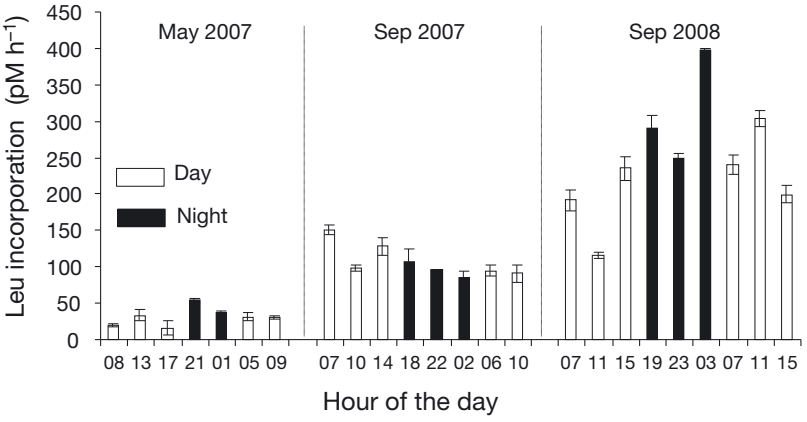

Fig. 1. Leucine incorporation during the 3 cruises conducted in Delaware coastal waters (±SD from technical triplicates)

also abundant, varying between about 9% (September 2007) and 11% (May). Cyanobacteria varied from 0.8% of all 16S rRNA sequences in May 2007 to 11.7% in September 2007 (Table 2). Actinobacteria, another abundant group in Delaware coastal waters, made up 7.3% in May and 8.5% in September 2007, but only 1.8% in September 2008 (Table 2). Rarefaction curves showed that our sequencing effort recovered about 65% of the total diversity (data not shown).

Expression of PR and 16S rRNA genes

The relative expression of SAR11 PR genes varied over the day–night cycle during the 3 cruises (Fig. 3). During the May cruise, the transcript to gene ratios of SAR11 PR genes (SAR11 PR mRNA:DNA ratio) peaked at 09:00 h both on Day 1 (ratio of 1.5 ± 0.1) and Day 2 (3 ± 0.03). Expression was slightly lower at 13:00 h (ratio of 1.3 ± 0.05). During all other hours of this cruise, the relative expression of SAR11 PR genes was very low (mRNA:DNA < 0.3) (Fig. 3A). In September 2007, a similar pattern was observed (Fig. 3B). The SAR11 PR mRNA:DNA ratio peaked at 10:30 h on Day 1 (2.4 ± 0.1) and Day 2 (2.5 ± 0.04). The relative expression at other times was lower, remaining below 0.75 during the day and night. In September 2008, the relative expression of SAR11 PR was again higher during the day than during the night (Fig. 3C). The relative expression was highest at 11:00 h during Day 1 (ratio 1.4 ± 0.3) and at 15:00 h during Day 2 (1.5 ± 0.03). During the night, SAR11 PR mRNA:DNA ratios were <0.6. Most of the change in the SAR11 PR ratios was due to changes in mRNA. During each cruise, PR gene abundance varied 3-fold, while PR mRNA concentration varied 7-fold (data not shown).

To compare changes in PR and 16S rRNA ratios, the data from all 3 cruises were combined and averages (±SE) were calculated for day and night (Fig. 4). During the 3 cruises, SAR11 PR mRNA:DNA ratios differed between day and night, but the ratio for SAR11 16S rRNA genes (16S rRNA:rDNA) did not (Fig. 4). The SAR11 PR mRNA:DNA ratio was 2.5-fold higher during the day (1.30 ± 0.30) than at night (0.46 ± 0.05). The difference was statistically significant (Student's *t*-test, $p < 0.05$). In contrast, the SAR11 16S rRNA:rDNA ratio did not change significantly between day (54 ± 15) and night (52 ± 8). The total bacterial 16S rRNA:rDNA ratio also did not vary significantly between day and night (Fig. 4).

Correlations between transcript to gene ratios and environmental parameters

The transcript to gene ratio for SAR11 PR and SAR11 16S rRNA varied about 30-fold and 20-fold,

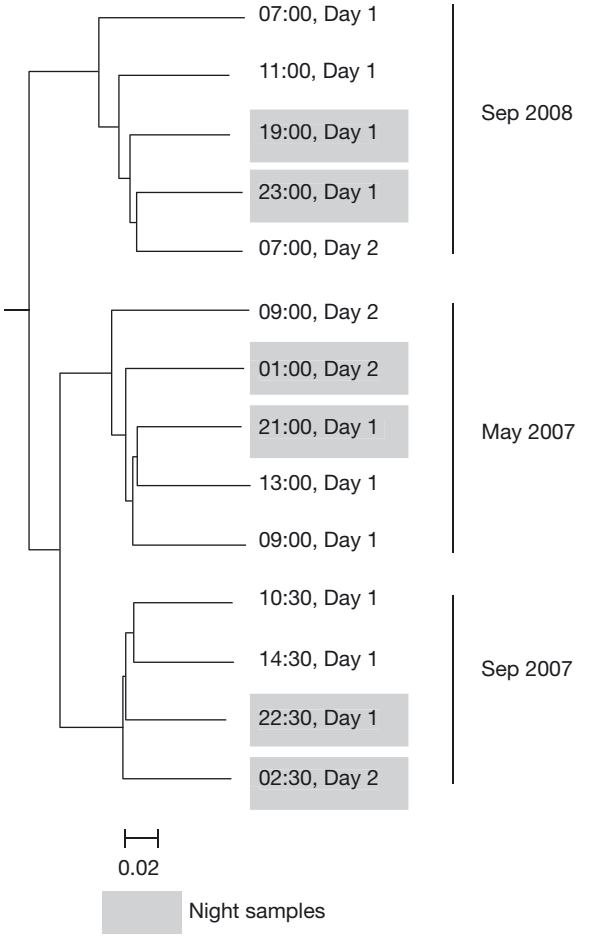

Fig. 2. UPGMA clustering of total bacterial communities defined by pyrosequences of 16S rRNA genes (97% of sequence similarity). Samples collected during the night are indicated with a gray background

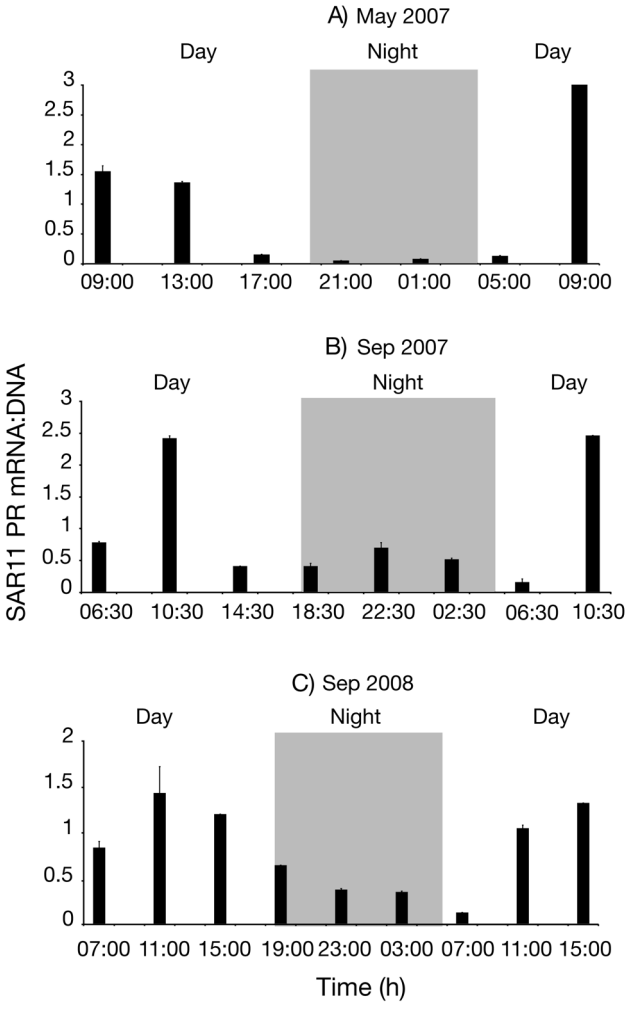

Fig. 3. SAR11 proteorhodopsins (PR) mRNA:DNA ratio (±SE from technical triplicates) over time in (A) May 2007, (B) September 2007 and (C) September 2008. Samples collected during the night are indicated with a gray background

respectively, but we did not find any significant correlation between the 2 ratios (Pearson's correlation coefficient, r = 0.3, p > 0.05, n = 24) (Fig. 5A). The relationship between SAR11 PR genes and total bacterial 16S rRNA genes was also not significant (r = 0.2, p > 0.05, n = 24, data not shown). However, there was a significant relationship between SAR11 16S rRNA:rDNA and the total bacterial 16S rRNA:rDNA ratio (r = 0.89, p < 0.01, n = 24) (Fig. 5B).

There was also a significant relationship between PAR and the mRNA:DNA ratio for SAR11 PR, excluding the night data (r = 0.66, p < 0.05 n = 15). In contrast, no significant correlation was found between the measured environmental parameters and the SAR11 PR mRNA:DNA ratios (r = 0.1 to 0.4, p > 0.05, n = 24; data not shown).

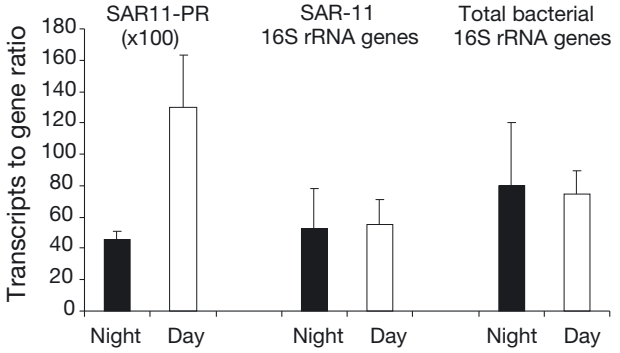

Fig. 4. Averages (±SE) of transcript to gene ratios for SAR11 proteorhodopsins (PR) (×100), SAR11 16S rRNA and total bacterial 16S rRNA during day and night in Delaware coastal waters. The data are from all 3 cruises

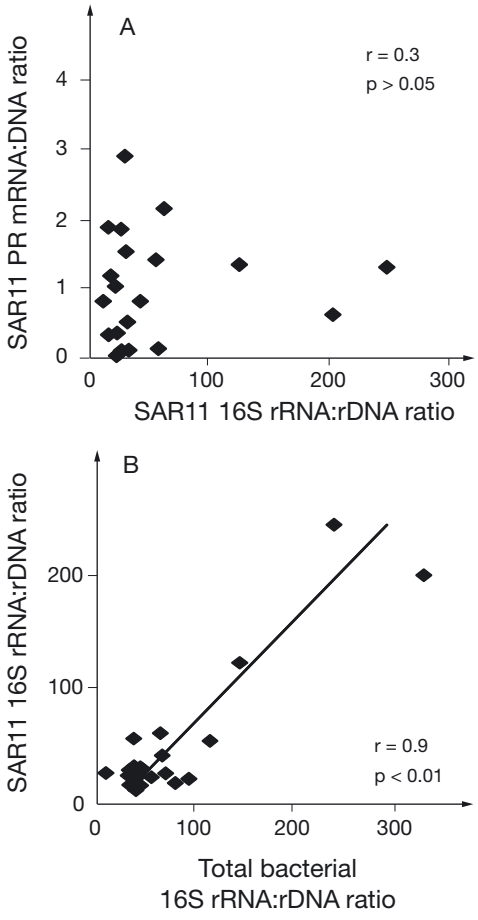

Fig. 5. (A) Relationship between the SAR11 proteorhodopsins (PR) mRNA:DNA ratio and the SAR11 16S rRNA:16S rDNA ratio. (B) Relationship between the SAR11 16S rRNA:rDNA ratio and the total bacterial 16S rRNA:rDNA ratio

DISCUSSION

In this study, we examined patterns of PR expression by the widespread SAR11 bacterial clade during 3 cruises in Delaware coastal waters. We found that the relative expression of SAR11 PR was about 2.5-fold higher during the day than at night and that the relative PR expression was modulated by the amount of PAR. Our experiment supports the existence of a tight relationship between light and relative PR expression in marine SAR11 bacteria, but there was no relationship between PR expression and 16S rRNA:rDNA ratios.

These field data are consistent with previous studies demonstrating higher PR expression with light in microcosm experiments with natural bacterial communities (Lami et al. 2009a) and pure culture experiments with the flavobacterial strain *Dokdonia* (Gómez-Consarnau et al. 2007, Kimura et al. 2011). In contrast, expression of PR by *Candidatus* Pelagibacter ubique HTCC1062, a cultivated representative member of the SAR11 clade, is higher in the dark than in the light (Steindler et al. 2011). This *Candidatus* P. ubique strain may not be representative of SAR11 strains found in Delaware coastal waters; it is well known that the SAR11 clade is phylogenetically and functionally diverse (Wilhelm et al. 2007). This diversity may explain the differences observed between PR expression by the *Candidatus* Pelagibacter strain in culture and by SAR11 cells in Delaware coastal waters. It also highlights the need for experiments with natural communities, such as those discussed here, and to integrate results from those experiments with appropriate data from lab experiments with model organisms.

Tides and coastal currents potentially complicate our interpretation of the temporal patterns in genes and transcription described in this study. However, several observations lead us to conclude that tides and currents do not explain the diel variation in the relative PR expression we observed. Physical and chemical data showed that variations between cruises were much larger than variations within a cruise. In spite of this variation, the influence of light on SAR11 PR and SAR11 16S rRNA transcription was similar during all 3 cruises in all 3 seasons. Clustering of total 16S rRNA gene sequences indicated that the bacterial community composition was highly similar in the water masses sampled during a single cruise. Even if communities varied among the water masses we sampled during a cruise, they appeared to respond similarly to daylight variations. We suspect that a similar physiological response to light occurred in all the SAR11 communities we sampled in Delaware coastal waters.

A key question addressed in this work is whether the expression of PR fuels growth-related activity of SAR11 bacteria in coastal waters. In this study, we used the relative expression level of 16S rRNA genes as a measure of this aspect of bacterial activity, an approach based on the relationship between rRNA levels per cell and growth rates and between rRNA synthesis and protein synthesis in pure cultures (see review by Blazewicz et al. 2013). Several previous studies have used this relationship to explore growth-based activity in natural communities (Blazewicz et al. 2013). In particular, Campbell et al. (2012) used 16S rRNA:rDNA ratios to monitor SAR11 growth-related activity as a function of several environmental variables at the same Delaware site as this study. We did not see a significant relationship between 16S

rRNA:rDNA and PR mRNA:DNA for SAR11, suggesting that energy from PR did not substantially affect SAR11 growth-related activity.

The lack of an obvious coupling between PR expression and the 16S rRNA:rDNA ratio for SAR11 is consistent with the theoretical calculations of Kirchman & Hanson (2013), who suggested that the amount of energy provided by PR is small compared with the energy required for bacterial growth. It would be difficult to detect a small contribution, especially with natural microbial communities. Light appears to enhance uptake of methionine and ATP by SAR11 cells in the North Atlantic Ocean by about 30% on average (Gómez-Pereira et al. 2013), which is more than predicted by theoretical calculations (Kirchman & Hanson 2013), but still near the detection limits of many methods. This level of enhancement may be too small to detect with 16S rRNA:rRNA ratios, especially if there is uncoupling between 16S rRNA synthesis and growth. Levels of 16S rRNA would also likely not capture any enhanced survival of bacteria due to PR, as observed for *Candidatus* P. ubique (Steindler et al. 2011). Light-enhanced survival of bacteria probably does not depend on enhanced protein synthesis and thus would not likely be detectable with 16S rRNA data.

Other environmental conditions have recently been shown to upregulate PR expression, but some of these conditions are likely not found in coastal waters, while others lead to a prediction opposite from the observed results. These conditions include the transition from the exponential to stationary phase and energy limitation caused by respiratory stress, both of which lead to higher PR expression in various cultivated bacteria (Gómez-Consarnau et al. 2010, Akram et al. 2013). In contrast to the laboratory studies, we sampled well oxygenated, mesotrophic coastal waters where bacteria likely grow continuously without respiratory stress. While bacterial growth is likely limited by organic carbon in these waters, the laboratory experiments showing higher PR expression during energy limitation would lead us to expect PR expression to be lower, not higher during the day, when release of labile organic material from phytoplankton is thought to be highest (Nagata 2000). Thus, an indirect effect of light on SAR11 PR expression appears unlikely.

The complicated relationships among PR, bacterial growth and light have implications for understanding diel patterns in heterotrophic bacterial properties. Although diel patterns in cyanobacteria are well known (Vaulot et al. 1995), they are less clear cut for other members of the bacterioplanktonic community. Some studies have not found diel patterns in the incorporation of thymidine, leucine or bromodeoxyuridine (Riemann & Søndergaard 1984, Torreton & Dufour 1996, Gasol et al. 1998, Pernthaler & Pernthaler 2005). Other studies, however, have shown an effect of solar radiation on diel cycles of thymidine and leucine incorporation (Jeffrey et al. 1996, Ruiz-González et al. 2012a), bacterial division deduced from *ftsZ* expression (Yao et al. 2011) and *recA* gene expression (Booth et al. 2001) by natural bacterial communities. The inconsistency in diel patterns for bacterial communities may in part result from variation in bacterial community composition and in how different PR-bearing bacteria respond to light. Communities with a large number of *Flavobacteria* may be more responsive to light than those dominated by SAR11, based on the documented light effects on PR-bearing *Flavobacteria* versus cultured representative members of the SAR11 group.

Our field data indicate light-dependent expression of PR genes by SAR11 bacteria in natural microbial communities. These *in situ* data contribute to the debate about the relationship between light, activity and PR expression, which has been examined so far with microcosm or culture-based experiments. These results highlight the need to better understand and characterize the role of PR in microbial physiology and oceanography.

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