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REVIEW

# A Reduction of Transcriptional Regulation in Aquatic Oligotrophic Microorganisms Enhances Fitness in Nutrient-Poor Environments

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**SUMMARY** In this review, we consider the regulatory strategies of aquatic oligotrophs, microbial cells that are adapted to thrive under low-nutrient concentrations in oceans, lakes, and other aquatic ecosystems. Many reports have concluded that oligotrophs use less transcriptional regulation than copiotrophic cells, which are adapted to high nutrient concentrations and are far more common subjects for laboratory investigations of regulation. It is theorized that oligotrophs have retained alternate mechanisms of regulation, such as riboswitches, that provide shorter response times and smaller amplitude responses and require fewer cellular resources. We examine the accumulated evidence for distinctive regulatory strategies in oligotrophs. We explore differences in the selective pressures copiotrophs and oligotrophs encounter and ask why, although evolutionary history gives copiotrophs and oligotrophs access to the same regulatory mechanisms, they might exhibit distinctly different patterns in how these mechanisms are used. We discuss the implications of these findings for understanding broad patterns in the evolution of microbial regulatory networks and their relationships to environmental niche and life history strategy. We ask whether these observations, which have emerged from a decade of increased investigation of the cell biology of oligotrophs, might be



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relevant to recent discoveries of many microbial cell lineages in nature that share with oligotrophs the property of reduced genome size.

**KEYWORDS** marine microbiology, metabolic regulation, transcriptional regulation

## INTRODUCTION

Microbial cells monitor intracellular and extracellular variables and respond to detected changes using regulation. Regulation governs intercellular interactions (e.g., quorum sensing), exploits or protects the cell from environmental conditions (e.g., catabolic operons and stress responses), and modulates internal processes to achieve homeostasis across growth cycles (e.g., the control of cellular growth and division). Regulatory mechanisms of microbial cells have been explored at length, with the consensus that bacteria generally respond to environmental stimuli via transcriptional regulation (expressing or repressing genes). However, there are many additional layers of regulation in bacterial cells: regulation mediated by structural RNAs (e.g., riboswitches), mechanisms that modulate mRNA translation (e.g., DEAD box ATPases), covalent and allosteric posttranslational modifications of proteins (e.g., acetylation of proteins), and internal regulation of cellular metabolism based on the kinetic properties of enzymes (e.g., partitioning of glucose and galactose metabolism). Most of what we know about microbial regulation has been derived from the study of cells that can easily be cultured, although significant work has been done on regulation in extremophiles and the broader uncultured microbial diversity.

The study of microbial regulation is relevant beyond the study of cell physiology. On an ecological scale, regulation in response to environmental changes and fluctuations in cell physiology determines which genes are turned on and off at a given point in time, for example impacting which organic compounds are oxidized. Thus, the mere presence of a gene in the environment does not mean that the protein coded by that gene is produced. By grasping how cells in the ocean regulate protein production, we might increase our ability to predict carbon oxidation functions vital to understanding the global carbon cycle. Understanding regulatory responses of microbes to fluctuating environments is also critical in a variety of other fields, including in plant-microbe interactions, the study of virulent bacteria, and biotechnological settings, as summarized in a recent article (1).

Prokaryotes are generally divided into two categories based on lifestyle strategy: oligotroph or copiotroph, with a broad spectrum between the two opposites. These two strategies are also relative measures of lifestyle based on the magnitude of nutrient fluxes and concentrations in environments; for instance, even the highest concentrations of nutrients in the ocean are lower than concentrations regularly experienced by gut bacteria. Oligotrophic microorganisms are adapted to thrive in environments with very low nutrient fluxes ($<$0.1 mg of C/L per day) and achieve maximal growth rates under these conditions (2); high-nutrient concentrations are known to inhibit growth of oligotrophs (3, 4). Copiotrophs are opportunists, only experiencing maximal growth rates at high nutrient concentrations, but are capable of surviving at low nutrient conditions for long periods of time, essentially in a state of starvation (5–8). The oligotroph/copiotroph dichotomy is a simplistic classification of microbes: many microorganisms fall somewhere in between, with varied combinations of characteristics associated with the two extremes (9). Because of this, various other frameworks have been proposed, such as the competitive/stress tolerator/ruderals triangle (C-S-R) (9–11), among others (12, 13), which provide useful insights into microbial ecosystem functioning. Despite this, the oligotroph/copiotroph dichotomy benefits from its simplicity, providing simple categories that broadly assess the niches and physiologies of microbes and ease communication among researchers.

Overall, there are more oligotrophic bacteria in the world than copiotrophic bacteria, primarily because the majority of the world is covered by oligotrophic ocean environments (14, 15). The two most abundant clades of bacteria in the world are also its

most well-studied oligotrophs: SAR11 and *Prochlorococcus* (16, 17). *Prochlorococcus* is a group of very small (cell volume, ~0.14 $\mu m^3$) photosynthetic cyanobacteria that are ubiquitous in the world's oceans between 40°S and 40°N latitudes and have a minimal genome (18–20). They are the most abundant type of phytoplankton in the world, with an estimated $2.9 \pm 0.1 \times 10^{27}$ cells worldwide (21). There are several reviews of *Prochlorococcus*, including a recent, highly relevant review of regulation in *Prochlorococcus* (18, 19, 22). SAR11 is a clade of heterotrophic alphaproteobacteria that are very small (cell volume, 0.01 $\mu m^3$), ubiquitous in the world's oceans at high densities (especially at the surface), are nonmotile, and have one of the smallest genomes of any free-living organism (17, 23). Globally, it is estimated that there are $2.4 \times 10^{28}$ SAR11 cells in the world (17). As with *Prochlorococcus*, there are several helpful SAR11 reviews, including a 2017 general review of SAR11 and a review of genome streamlining in SAR11 (24, 25).

The oligotroph/copiotroph life history strategies are theorized to be associated with a variety of physiological and genomic adaptations, with oligotrophs generally thought of as being small in cell size, free-living, nonmotile, and slow-growing and having streamlined genomes with low numbers of rRNA gene operons and low GC content (2, 14, 15, 23, 24, 26–30). Other studies have found a lack of correlation between assigned lifestyle strategy and proposed phenotypic traits across a range of environments (31, 32). In uncovering broad associations between phenotypic traits and lifestyle strategy, there are two difficulties: first, most cultured microbes are copiotrophs, due to the ease of isolating and culturing them (15, 27, 33, 34); second, microbes are often misclassified as oligotrophs based solely on their presence in oligotrophic environments. For instance, *Caulobacter* has long been studied as a model aquatic oligotroph (see, for example, reference 35); however, a recent study has shown that *Caulobacter* microbes are primarily found in high-nutrient soils and use aquatic systems mainly for dispersal (36), casting doubt on their reputed character as aquatic oligotrophs.

In this review, we examine one of the most salient examples of a theorized broad association between regulatory strategies and life history strategies. The comparative study of microbial regulation is not a "field" in the sense that you will find symposia devoted to the topic. Rather, we tend to see each cell type as an amalgam of regulatory functions that suits its needs, and we do not often consider whether cellular regulatory strategies can be divided into broad categories that fit modalities in lifestyle. For each type of regulation known to operate in prokaryotic organisms (transcriptional, posttranscriptional, posttranslational, and kinetic), we first briefly introduce its utility to cells, describe its mode of action, review the evidence (if any) for different uses between copiotrophs and oligotrophs, and finally provide examples of each type of regulation in aquatic oligotrophs. We show that the evidence indicates a reduction in transcriptional regulation in oligotrophs, resulting in constitutive expression of many genes (Fig. 1). Many posttranscriptional levels of regulation seem to be present at similar levels in oligotrophs as in copiotrophs, but they take on added importance to the functioning of cells in the absence of transcriptional regulation.

Finally, we ask why copiotrophs and oligotrophs might use a common set of regulatory mechanisms with very different preferences on how, and how frequently, they are employed. We ask whether these differences are part of a multivariate continuum of cell properties, such that theorizing mainly serves a heuristic role, or whether there are trends in evolution that can lead different lineages to converge on a set of cell regulatory properties associated with oligotrophy that are distinctly identifiable and uniquely different from those associated with copiotrophy. We ask how creating such categories might help us understand the ecology and physiology of cells and whether it makes sense to develop broad rules that could be used to define these categories.

## REGULATION AT THE TRANSCRIPTIONAL LEVEL

The central dogma of molecular biology states that genes, encoded in DNA, are transcribed into mRNA and then translated into protein. Cells can regulate the amount of a given protein in the cell by modulating all parts of this process. Transcriptional

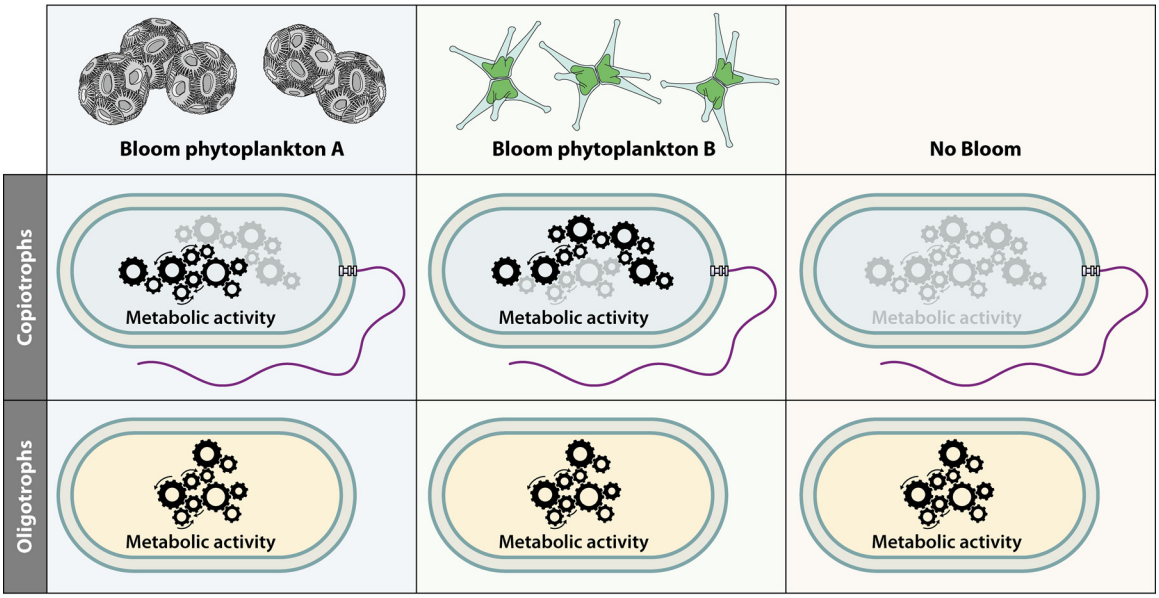


**FIG 1** Illustration of the primary hypothesis explored in this article. Aquatic oligotrophic microorganisms are depleted in transcriptional regulation compared to copiotrophs. This results in constitutive expression of most of their genes, no matter the current nutrient regime they are experiencing. This constitutive expression may further be modified by posttranscriptional, posttranslational, or kinetic/metabolic regulation, but the result remains that a majority of proteins from oligotrophic genomes are expressed most of the time. "Metabolic activity" here refers to all levels of regulation, from transcriptional to posttranslational.

regulation has the benefit of saving on cellular resources when a nutrient is unavailable, with no excess production of mRNA or protein. The downside is that it can be relatively slow (on the order of minutes) to respond to a stimulus (37) and requires extra regulatory machinery. In *Escherichia coli*, it has been shown that the majority of protein expression changes are attributable to transcriptional regulation (38).

## Introduction to Transcriptional Regulation

Regulation at the transcriptional level modulates the amount of a given gene being expressed at any given time. Generally, aquatic microbes are thought to respond to environmental changes via a sense-response system, where an environmental stimulus initiates a transcriptional response in the cell (39). These transcriptional responses can take multiple forms.

Suites of genes that are regulated together in response to a stimulus are called regulons; regulons are generally regulated by a combination of transcription factors (40), proteins that either enhance or repress the binding of RNA polymerase to the promoter of a gene, and sigma factors ($\sigma$-factors) (41). $\sigma$-Factors are transcription initiation factors that are required for the binding of RNA polymerase to gene promoters. By having different $\sigma$-factors that bind to different gene promoters, cells can change expression of large sets of genes by simply changing which $\sigma$-factor is expressed (42). $\sigma$-Factors are usually used to modulate responses to large environmental changes, such as starvation, heat stress, etc. (43). *E. coli* cells have seven $\sigma$-factors, while some *Verrucomicrobia* have more than 30 $\sigma$-factors.

Transcriptional regulation can also happen in a more targeted way, through two-component regulatory systems, which translate environmental stimuli into a targeted regulatory response using phosphotransferase systems (44). Two-component systems are comprised of a histidine kinase, which senses the environmental stimulus, and a response regulator protein, which receives the signal from the kinase and activates the transcriptional response (45). The breadth of possible transcriptional regulation in bacteria, such as the types of transcription factors, $\sigma$-factors, and two-component systems, is extensive and has been reviewed before (40, 44, 46–48).

**Relevance of Transcriptional Regulation to Oligotrophs**

Oligotrophs have less transcriptional regulation than would be expected from their genome sizes. This has been demonstrated both bioinformatically by mining oligotroph genomes for genes associated with regulation, as well as experimentally, looking at changes in transcription in response to environmental changes.

A reduction in the number of mechanisms for transcriptional regulation is evident at the genome level in the most-studied representative of the SAR11 clade, "*Candidatus* Pelagibacter ubique" strain HTCC1062, which only has two $\sigma$-factors and four two-component regulatory systems (23). In a comparison of $\sigma$-factors in bacteria to genome size, a variety of oligotrophs, including SAR11, *Prochlorococcus*, and the abundant oligotrophic gammaproteobacterial clades SAR86 and SAR92, were found to have fewer $\sigma$-factors than would be expected based on genome size (24). In a survey of two-component systems in marine bacteria, it was discovered that oligotrophy was associated with a reduced number of two-component regulatory systems, with oligotrophs having, on average, 0.2 histidine kinases per 100 genes, while copiotrophs had 1.4 (45). Similarly, the oligotrophic clade II of the globally abundant cyanobacteria *Synechococcus*, such as *Prochlorococcus*, has limited numbers of histidine kinases and response regulators (7 and 12, respectively) compared to the abundant freshwater, more copiotrophic *Synechocystis* cyanobacteria (47 and 42, respectively) (49). Within the *Dadabacteria* phylum, the marine pelagic subclade that exhibits genome streamlining has fewer genes associated with two-component systems and motility (50).

In an analysis of the genomic features that differentiate oligotrophs and copiotrophs, it was discovered that, compared to copiotrophs, oligotroph genomes were depleted in genes in all categories relating to regulation (14). These categories included $\sigma$-factors, transcriptional regulators (COG0583), AraC-type DNA-binding domain-containing proteins (COG2207), DNA-binding winged-helix–turn–helix (HTH) domains (COG3710), regulators with FOG:GGDEF domains (COG2199), and regulators with FOG:EAL domains (COG2200) (14). Another, more recent paper found a similar result (i.e., depletion of genes in the transcriptional regulation category in oligotrophs) when categorizing oligotroph/copiotroph based on maximal growth rates as predicted by codon usage bias (27). This definition of oligotroph/copiotroph is based on one cell property but has the advantage of being quantitative in nature. However, this functional analysis used genomes from the RefSeq database, which spans a variety of habitats. We recently repeated this analysis using a large collection of marine metagenome-assembled genomes (51) (Fig. 2). We first found that the genomes of the oligotrophic microbes in this data set generally conform to predicted genome characteristics of oligotrophs: smaller genomes and lower GC content (Fig. 2A and B). Next, as found in the previous analysis that used RefSeq genomes, copiotrophs were significantly ($P < 0.05$, Mann-Whitney test with Benjamini-Hochberg correction) enriched in genes for transcriptional regulators compared to oligotrophs (Fig. 2C), again confirming that oligotrophs are depleted in transcriptional regulation at the genomic level. Finally, a recent review compared traits of marine pelagic microbes (generally oligotrophs) to freshwater sedimentary microbes (generally copiotrophs) again found that the marine pelagic microbes tended to have smaller genomes, lower GC content, fewer $\sigma$-factors, and fewer two-component systems than the freshwater sediment microbes (52).

Several experimental studies have reported a global reduction of transcriptional regulation in oligotrophs. In a comparison of rRNA/rDNA ratios in two oligotrophic bacteria, SAR11 and SAR92, and two copiotrophic bacteria, *Roseobacter* and *Flavobacteria*, Lankiewicz et al. found that the two oligotrophs did not modulate their ribosome number in response to growth rate, indicating a lack of transcriptional regulation of rRNA in the oligotrophs compared to copiotrophs (53). In a subsequent paper, the same four organisms were compared for their global transcriptional changes in response to transitioning from exponential to stationary growth phases (54). In the two oligotrophs, the change in transcript abundances between exponential and stationary phases was extraordinarily minimal, with almost all changes falling below a $\log_2$-fold change of 2 (54). In the copiotrophs, on the other hand, transcript abundances varied widely

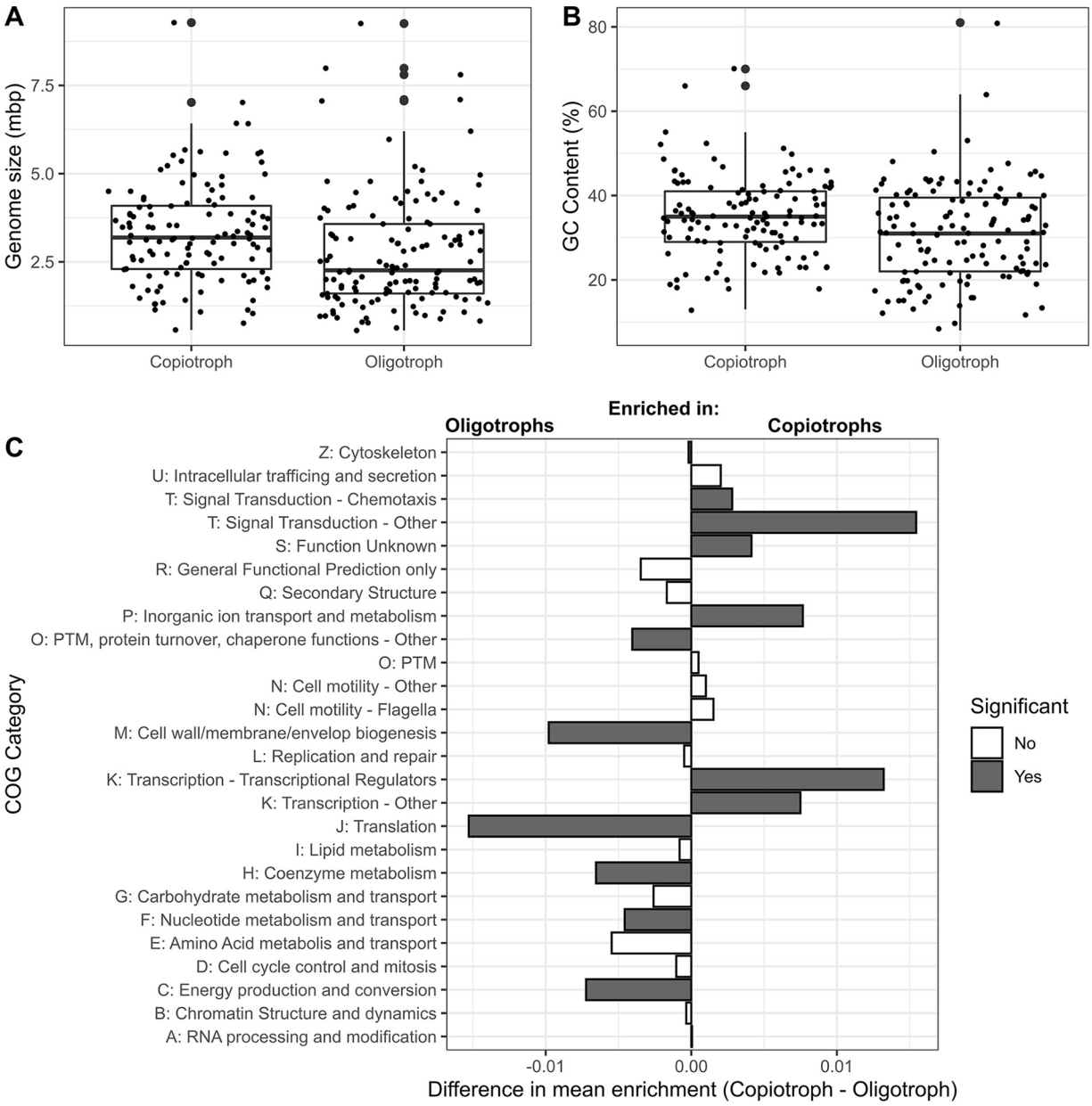


**FIG 2** Analysis of marine copiotroph/oligotroph metagenome assembled genomes (MAGs) from Tully et al. (51), with lifestyle strategy defined by predicted maximal growth rate based on codon usage patterns, as in Weissman et al. (27). (A and B) Comparison of genome characteristics of the two lifestyle categories from this data set. (C) Enrichment of different COG categories in copiotrophs or oligotrophs. (For full methods for this analysis, see reference 76.) Category T was split between COG categories having "chemotaxis" in their description and all others. Category N was split between categories with "flagella" in their description and all others. Category K was split between categories with "transcriptional regulator" in their description and all others. Category O was split between categories representing common posttranslational modifications (acetylation, phosphorylation [kinases], ubiquitination, methylation, glycosylation, adenylation, and peptidases) and all others. (The figure was adapted from reference 76.)

between the two growth stages, with $\log_2$-fold changes reaching 10 (54). The low amplitude changes in SAR11 likely reflect the absence of the stationary-phase sigma factor $\sigma^S$ in these cells, which results in a muted proteomic response to entering stationary phase (55). Instead of wholesale proteome remodeling, modest increases in the abundance of proteins involved in maintaining cellular protein pools, such as chaperones, signal transducing proteins, and amino acid synthesis enzymes (especially methionine and cysteine) were reported in SAR11 cells entering stationary phase (55). Sowell et al. argued that the muted proteomic response of "*Ca.* Pelagibacter ubique" allows

them to cope with short periods of nutrient deprivation and resume growth quickly, making a comprehensive global stationary-phase response unnecessary (55).

Studies of *Prochlorococcus* using cultures, metatranscriptomics, and pigment analyses point to control of chlorophyll production by protein-level, not transcriptional, regulation (56, 57). In a proteomic analysis of the oligotrophic alphaproteobacterium *Sphingopyxis alaskensis* in glucose-limited chemostats, only 12 of over 1,000 resolved proteins were found to be significantly different in abundance compared to nutrient-replete conditions (58). Similarly, the oligotroph *Sphingomonas* sp. strain RB2256 showed consistent growth rates across low and high nutrient conditions, which was hypothesized to be due to constitutive expression of protein-synthesizing machinery (59). In a mesocosm experiment where surface marine microbial communities were exposed to deep-see water, the highly abundant oligotrophs (SAR11 and *Prochlorococcus*) showed little to no transcriptional response, compared to the copiotrophic *Alteromonas* species detected (60).

There is additional experimental evidence indicating that oligotrophs have limited transcriptional regulation at the global level. Much of this evidence comes from studies that compare data from transcriptomic (total RNA from cells that is sequenced [61]) and proteomic (total proteins from cells that are analyzed via mass spectrometry [62]) studies. By simultaneously comparing changes in transcript and protein abundance for genes, the regulatory control of that gene can be examined (63). If a gene is under transcriptional control, one would expect changes in mRNA and protein abundance to be correlated for that gene, while genes under posttranscriptional regulation would, in principle, have decoupled protein and transcript abundance. However, differences in the stability of mRNA and proteins in cells can also skew correlations (63). As an example, in *E. coli*, the half-life of mRNA molecules is only minutes long, while proteins have an average half-life of 20 h, resulting in a lack of correlation between transcripts and proteins on a single-cell basis (64). However, in general, when transcript and protein abundances are averaged over a population, there is a linear correlation, with at least 40% of variation in protein abundance being explained by transcript abundance (38, 65). While these findings point to posttranscriptional regulation being widespread across all domains of life, they also indicate that, in general, protein and transcript abundances are correlated (66). In three papers examining transcriptional and proteomic responses in SAR11 to iron, sulfur, and nitrogen limitation, there was no correlation between transcript and protein abundances, except for a few genes, indicating a lack of transcriptional regulation at a global level in SAR11 (Fig. 3A) (67–69). Similarly, in an examination of diel oscillations in *Prochlorococcus* transcripts and proteins, transcript abundances were found to vary much more widely than protein abundance for genes, signifying heavy posttranscriptional regulation (56). In several of these papers, the possibility of either mRNA degradation or missed pulses of mRNA skewing transcript abundances was tested, but no evidence was found to support these possibilities (56, 68). On the environmental level, one study that paired metagenomic and metaproteomic data from oligotrophic ocean samples found that the most abundant members of the microbial community (SAR11, *Prochlorococcus*, *Synechococcus*, and SAR116) had strong coupling between the presence of a gene in the genome and the corresponding protein being expressed, while the opposite was true for the rarer, copiotrophic members of the community (70). This suggests that oligotrophs were expressing most of their genes in the samples studied, while copiotrophs were not.

When examining a trove of published and unpublished proteomic data collected on HTCC1062 cells over a variety of nutrient limitation conditions, it becomes clear that these cells constitutively express the majority of their genes. Rarefaction curves of the proteomic coverage of HTCC1062 shows almost 80% coverage due to the constitutive expression of most genes (Fig. 4). In contrast, the nonoligotrophic cyanobacterium *Synechocystis* PCC68803 proteome only shows around 50% coverage, indicating that only about half of the genes in this organism are being expressed under a given set of conditions (Fig. 4). This has been borne out in other oligotrophs as well, including a cultured representative of the ammonia-oxidizing *Thaumarchaeota* phylum, *Nitrosopumilus*

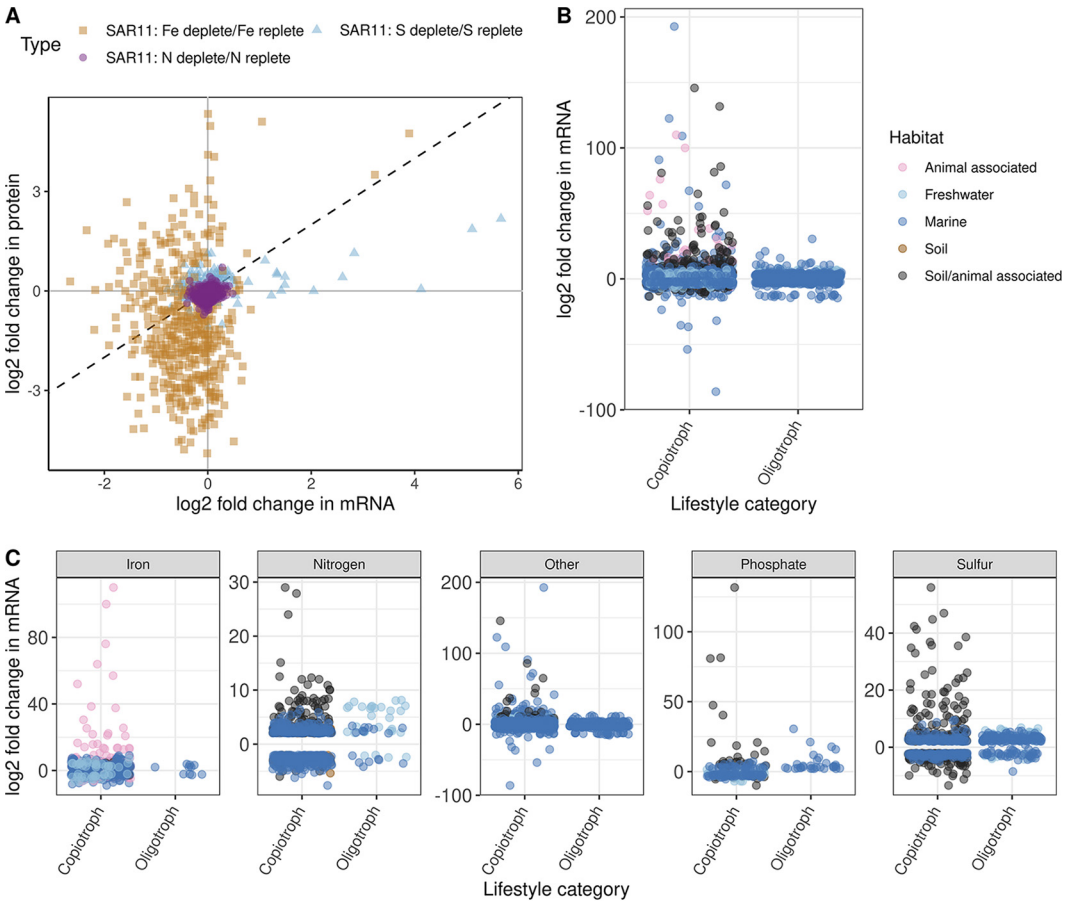


**FIG 3** (A) The prevalence of posttranscriptional regulation in SAR11 cells is apparent when comparing transcript and protein abundances ($log_2$-fold) in SAR11 cells under a variety of nutrient limitations. The dashed line indicates a 1:1 correlation between transcript and protein abundance, which the data clearly does not fit. Data were digitized from previous publications (67–69) using WebPlotDigitizer v4.6 (189). (B and C) Comparison of $log_2$-fold changes in transcript abundance in copiotrophs or oligotrophs in response to a variety of environmental challenges. Aggregated data are shown in panel B and are broken out by nutrient limitation state in panel C. The following species are represented for iron limitation: for copiotrophs, *Pseudomonas fluorescens* (190), *Listeria monocytogenes* (191), *Alteromonas macleodii* (192), *Campylobacter jejuni* (193), *Synechocystis* (194), *Pasteurella multocida* (195), and *Synechococcus* sp. strain PCC 7002 (196); for oligotrophs, *Prochlorococcus* (141) and "*Ca.* Pelagibacter ubique" (67). The following species are represented for nitrogen limitation: for copiotrophs, *Pseudomonas putida* (197), *Synechococcus elongatus* PCC7942 (198), *Mycobacterium smegmatis* (199), and *E. coli* (77); and for oligotrophs, *Dehalococcoides ethenogenes* (200), *Prochlorococcus* (201), and "*Ca.* Pelagibacter ubique" (68). The following species are represented for phosphate limitation: for copiotrophs, *E. coli* (78), *Rhodobacter sphaeroides* (202), and *Synechococcus* sp. strain PCC 7002 (196); and for oligotrophs, "*Ca.* Pelagibacter ubique" (94). The following species are represented for sulfur limitation: for copiotrophs, *Pseudomonas aeruginosa* (203), *Synechococcus* sp. strain PCC 7002 (196); and for oligotrophs, *Emiliania huxleyi* (204), *Arthrospira* (205), "*Ca.* Pelagibacter ubique" (69). The following species are represented for other environmental challenges: for copiotrophs, *Bacillus subtilis* (superoxide stress) (206), *Marinobacter hydrocarbonoclasticus* (hydrocarbon exposure) (207, 208), *Alcanivorax borkumensis* (hydrocarbon exposure) (209), *Alteromonas naphthalenivorans* (contaminated seawater exposure) (210), and *Synechococcus* sp. strain PCC 7002 ($CO_2$ limitation) (196); and for oligotrophs, OM43 (variety of nutrient exposures) (86), *Prochlorococcus* (coculture with heterotroph [211]; $CO_2$ limitation [212]), and "*Ca.* Pelagibacter ubique" (DMSP versus methionine exposure [74]). A threshold $log_2$-fold change of 2 and −2 was used to reduce the amount of data; for "*Ca.* Pelagibacter ubique" nitrogen limitation and DMSP exposure, this threshold removed all transcripts. For species with a murky lifestyle strategy, their maximal growth rate reported in literature was used for classification as described previously (27). Not all data from these papers was included for ease of visualization, but the largest changes in transcript abundances were selected.

*maritimus* SCM1, which is ubiquitous in oligotrophic waters and has a small genome (71). The vast majority of genes in these cells were also constitutively expressed in exponentially growing cells (72).

In many studies of SAR11 and other heterotrophic oligotrophs, the data have pointed to a lack of transcriptional regulation of genes involved in carbon metabolism. One of the easiest ways to test for this is to compare the uptake rates and/or metabolic rates of carbon compounds in naive cells (i.e., cells grown in the absence

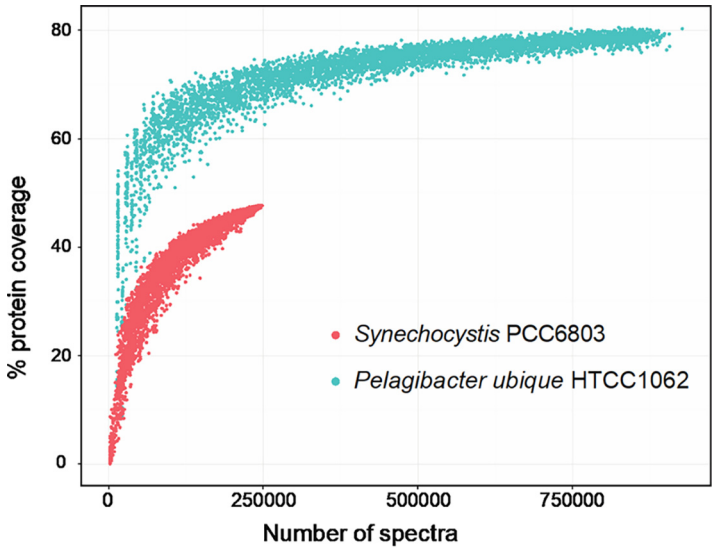


**FIG 4** Rarefaction plots for proteomic coverage for "*Ca.* Pelagibacter ubique" HTCC1062 (oligotroph) compared to that of the copiotrophic *Synechocystis* PCC6803. For HTCC1062, protein expression data from a range of lab experiments across a variety of conditions (nutrient depletions, stationary phase, etc.) were uploaded to the Proteomics Identifications database (PRIDE). The coverage of the predicted cellular proteins in HTCC1062, and the numbers of spectra measured for these peptide fragments were compared to a copiotrophic organism (*Synechocystis*) that had undergone lab-based experiments from a similar set of conditions and whose proteomics measurements had been done on a similar type of mass spectrometer, to allow for direct comparisons. The reduction in transcriptional regulation found in HTCC1062 is correlated with a highly conserved (and thus well-covered) proteome, with the majority of genomic proteins having a high percentage of coverage at high peptide spectra levels.

of a compound of interest) and preconditioned cells (i.e., cells grown with the compound of interest for a reasonable amount of time). If naive and preconditioned cells have the same uptake and/or metabolic rates, then the reasonable conclusion is that the genes required for uptake and/or metabolism of that compound are constitutively expressed at the same level in the presence and absence of that compound. In SAR11 for dimethylsulfoniopropionate (DMSP), dimethyl arsenate (DMA), and L-alanine, naive and preconditions cells were found to have the same uptake and/or metabolic rates (73–76). Conversely, if there is a significantly larger uptake and/or metabolic rate of the compound in preconditioned cells, one can conclude that the cell has the genes required for uptake and/or metabolism under transcriptional regulation. In a study comparing regulation of L-alanine metabolism in SAR11 and a marine copiotroph, *Alteromonas macleodii*, the copiotroph was found to have a significantly larger metabolic rate of L-alanine in preconditioned cells compared to naive cells (76).

### Examples of Transcriptional Regulation in Oligotrophs

Oligotrophic bacteria do use transcriptional regulation to respond to some environmental stimuli, but many of these responses (as described below) are significantly smaller in magnitude than commonly observed transcriptional responses in copiotrophs. For instance, the maximum $\log_2$-fold change in transcripts found in nitrogen-starved *Pelagibacter* cells was 1.5 compared to 15 in nitrogen-starved *E. coli* (77); the maximum $\log_2$-fold change in transcripts in phosphate-limited *Pelagibacter* cells was 30 compared to 131 in phosphate-limited *E. coli* (78). This is illustrated on a broad scale in Fig. 3B and C, which shows that, in response to the same types of nutrient limitation, oligotrophs have a highly muted transcriptional response compared to copiotrophs. Poindexter theorized that oligotrophs would have low-magnitude regulatory responses, since a large regulatory response would be out of proportion to the minimal nutrient concentrations they are regularly exposed to (79). A common observation in oligotroph biology is that growth rates rarely accelerate in response to positive

environmental changes. This factor might mute the apparent intensity of positive transcriptional changes when comparisons are made to copiotrophs.

In a study of diel transcript abundances in the oligotrophic open ocean, Ottesen et al. found significant diel changes in transcripts in a variety of oligotrophic bacteria, including *Prochlorococcus*, SAR11, SAR86, and SAR116 (80). However, the number of transcripts showing diel changes and magnitude of those changes were lower in the heterotrophic oligotrophic groups of organisms than the copiotrophs (80). Some of the changes in transcript levels may also be due to growth dilution, i.e., as cells grow or shrink, all cellular components must increase or decrease to some extent with changes in biomass, as observed in *E. coli* (38). *Prochlorococcus*, as a photoautotroph, showed strong, diel transcriptional responses that matched light levels (80). In a similar study of transcriptional changes in coastal and open-ocean microbes, several heterotrophic oligotrophs, including SAR11 and SAR86, were found to have significant transcriptional changes on a diel cycle (81). In contrast, a similar examination of diel transcriptomes in a coastal region did not find significant diel changes in gene expression in the heterotrophic oligotrophic groups, including SAR11 and SAR86 (82). There were, however, pathway-level, covariant changes in transcript abundances in these heterotrophic oligotrophic groups; as an example, in SAR11, ribosomal proteins and oxidative phosphorylation had a strong positive correlation with each other and a negative correlation with several transport gene transcripts (82). Interestingly, in two separate experiments, there were found to be diel, synchronous changes in transcript abundance between SAR11 and SAR86, which could either be due to the two groups responding to similar environmental signals or could be indicative of interspecies interactions (81, 82). Upon addition of dissolved organic matter (DOM) derived either from *Prochlorococcus* cultures or high molecular-weight DOM from surface waters to a surface microbial community, a swift (within 2 h) transcriptional response was observed in both SAR11 and *Prochlorococcus* cells (83). In both oligotrophs, genes involved in assimilating organic N compounds were upregulated, in addition to protein biosynthesis genes (83). Similarly, in response to polyamine addition to surface water mesocosms, SAR11 transcripts for genes involved in polyamine metabolism increased in abundance within hours of polyamine addition (84).

In cultures of oligotrophs, transcriptional responses to changing growth conditions or nutrient limitation have been observed. In cells from the oligotrophic methylotrophic clade OM43, which have highly streamlined genomes (85), strong changes in transcript abundance were observed when cells transitioned from exponential to stationary growth phase and in response to addition of specific nutrients (86). Genes involved in C metabolism showed the strongest response, including ribulose monophosphate, proteorhodopsin, and methanol dehydrogenase (86). *Nitrosopumilus maritimus* SCM1 cells have been found to downregulate genes involved in ammonia transport and metabolism (*amoA*, *amoB*, *nirK*, and *amtB*) upon ammonia limitation, while the *hsp20* gene for the molecular chaperone protein was upregulated (72, 87). This finding has been observed in other cell types undergoing stress, including SAR11 (67–69, 72, 88). Overall, however, a large proportion of genes are constitutively expressed by these *Thaumarchaeota* and the transcriptional response to ammonia limitation is relatively muted (71, 72, 87, 89).

In SAR11 cells, the regulatory response to inorganic nitrogen limitation was found to rely on a much simpler system for ammonium assimilation than the $P_{II}$ signal-transducing system that is found in most proteobacteria, with five fewer genes than other free-living alphaproteobacteria. This system in SAR11 cells is under transcriptional control of the two-component system NtrX/NtrY; transporters for organic nitrogen compounds, such as amino acids, opines, and taurine, were also upregulated in N-depleted SAR11 cells (68). Smith et al. speculated that the absence of the $P_{II}$ system in SAR11 contributes to genome streamlining at the cost of making these cells vulnerable to metabolic disruptions caused by competition for the metabolic intermediate 2-oxoglutarate, which is an intermediate both in ammonium uptake and in energy metabolism (68). All *Prochlorococcus* strains appear to have active versions of the $P_{II}$ regulatory

system, in addition to the global nitrogen regulatory protein NtcA (22, 90). However, these proteins show unique regulatory responses to N in *Prochlorococcus*: the $P_{II}$ system may not be fully phosphorylated, the urease enzyme is constitutively expressed, and the response of NtcA to N limitation is not as strong as in other cyanobacteria (91). Methylotrophic cells from OM43 also encode the $P_{II}$ regulatory system; under nitrogen limitation, these cells were found to have large transcriptional responses ($log_2$-fold changes above 10 in transcript levels) (86).

When SAR11 cells were iron limited, the ABC transporter for iron, SfuABC, was found to be highly upregulated, both in transcript and protein abundance, but other transcriptionally upregulated genes involved in iron metabolism were not translated (67). The abundance of the RNA chaperone CspL increased dramatically in the iron limited *Pelagibacter* cultures, whereas the paralog CspE declined, leading Smith et al. to speculate that *Pelagibacter* utilizes RNA chaperones (also know as DEAD-box ATPases) to control a global posttranscriptional regulatory response to iron limitation (67). RNA chaperones are found in all cell types and have been implicated in a variety of processes involving RNA, including RNA sequestration into subcellular structures (92), a process that can involve the recognition of RNA sequences by RNA chaperones. Smith et al. postulated that CspL might function by inhibiting the translation of nonessential transcripts until iron became available, although this hypothesis was not tested (67). However, RNA chaperones recently were implicated in the regulation of iron metabolism in *E. coli* (93).

Similar to iron limitation, when SAR11 cells experienced phosphate limitation, they vastly upregulated (30-fold) transporters for phosphorous-containing compounds (94), although this regulatory response was much lower than that observed in phosphate-limited *E. coli* cells (maximum fold change of 130) (78). However, there were large differences observed between the coastal ("*Ca*. Pelagibacter ubique" strain HTCC1062) and open ocean ("*Ca*. Pelagibacter" strain HTCC7211) strains of SAR11 studied, with the coastal strain upregulating its phosphate transporter and the open ocean strain upregulating its phosphonate (organic phosphate) transporter (94). HTCC7211 also upregulated genes for a C-P lyase (*phnGHIJKLM*), which cleaves phosphonate compounds into inorganic phosphate and methane (94). The transcriptional response in the coastal strain, HTCC1062, appears to be due to the upregulation of general stress-response genes such as *recA*, *lexA*, and *umuD*, but the transcriptional regulatory mechanism in the open ocean strain, HTCC7211, is not fully understood (94). In cells from the OM43 clade, phosphate limitation resulted in a decrease in the phosphate-specific transport system gene, but the response was relatively muted (only changes of 2- to 5-fold) (86).

In SAR11 cells exposed to light and starved for carbon, 9.7% of coding sequences showed differential expression compared to cells in the dark, including upregulation of genes involved in oxidative phosphorylation (95). Three of the upregulated genes were found to be transcriptional regulators, with one being a putative ferric uptake regulator protein (Fur), known to bind to specific sequences (Fur-boxes) upstream of regulated genes (95). A search for relaxed fits to Fur-boxes showed potential binding sequences upstream of several of the genes upregulated in the light, implicating this Fur protein in the transcriptional regulatory response to light and C starvation in SAR11 (95). The presence of light-dependent proteorhodopsin pumps in SAR11, combined with the lack of large-scale transcriptional changes in SAR11 in response to light availability, indicates that SAR11 cells use their proteorhodopsin pumps to produce enough energy to survive until more organic carbon nutrients are available, instead of undergoing proteome remodeling leading to a dormant state (95, 96). SAR11 cells seem to focus on small cellular changes to promote homeostasis and survive periods without nutrients, allowing them to quickly start up metabolism and growth when they encounter nutrient patches (25).

## REGULATION BY NONCODING RNA

Gene regulation via the use of noncoding RNA molecules mainly differs from transcriptional regulation in that it generally regulates genes after transcription of the

target gene has occurred and is carried out by an RNA molecule, not a protein (97). RNA-based regulation provides a faster response to environmental stimuli that comes at a lower genomic cost than protein-based regulation but usually does not result in changes in protein abundance that are as large of a magnitude as transcriptional regulation (24, 98). RNA-based regulation encompasses riboswitches, small RNA molecules, and CRISPR RNAs, the last of which will not be discussed here, since oligotrophs rarely have CRISPR arrays (99).

### Introduction to Riboswitches and Small Noncoding RNAs

Riboswitches are structural mRNA elements, usually located in the 5′ untranslated region of the RNA, that act in a *cis*-regulatory way, causing the early termination of transcription which either prevents translation of the gene it is present in (type 1) or causes mRNA degradation (type 2) (100). There are numerous reviews on riboswitches (100–103), but some relevant highlights are presented below. In both cases, the binding of a regulator molecule to the riboswitch changes the riboswitch conformation, which results in the regulatory outcome (101, 104). Another method of riboswitch action involves modulating the stability of the mRNA molecule, with ligand binding exposing an RNase-sensitive site (105, 106). Riboswitches are comprised of two domains, an aptamer domain and an expression platform (107). The regulatory ligand (aptamer) binds to the aptamer domain, which is highly conserved within classes of riboswitches (107). The expression platform is much more variable in sequence and is the portion that changes structure in response to the binding of the molecule to the aptamer domain (107). Many riboswitches act more as "dimmers" than on/off switches, with a gradient of responses to physiochemical factors, such as pH and temperature, as the stability of the riboswitch domain changes at a given physiochemical state (108, 109). There are currently at least 40 known classes of riboswitches, with many more likely to be discovered (102, 110, 111).

Small RNA regulators (sRNAs), or antisense RNAs, play critical regulatory roles in bacteria. For reviews of sRNA categories, functions, strategies for detection, etc. (112–116). The three primary categories of sRNAs are: *cis*-sRNAs, *trans*-sRNAs, and sRNAs that regulate proteins (117, 118). *cis*-sRNAs are located on the complementary strand from their regulatory target and carry out their regulation by binding directly to the mRNA produced from the gene and inhibiting translation (119). These *cis*-sRNAs usually regulate genes whose product is toxic to the cell at high concentrations (120). *trans*-sRNAs, in contrast, have limited complementation to the mRNA that they regulate, giving them a broader range of regulatory capacity, and are usually found at different genomic sites than the genes they regulate (114). Their mechanisms of action are also much broader, similar to riboswitches; binding of a *trans*-sRNA to a target mRNA can inhibit translation from occurring by occluding the ribosome binding site (Shine-Dalgarno sequence), which also often marks the mRNA for degradation (121, 122). They can also have positive effects on the target mRNA by relieving a secondary RNA structure in the mRNA, exposing the ribosome binding site (118). These sRNAs are often expressed during a specific physiological state and coordinate a cellular response to environmental stimuli (118). The binding of these *trans*-sRNA molecules to their target is often mediated by the ribosomal S1 protein, the Sm-like Hfq protein, or other RNA-binding proteins (123, 124). sRNAs that regulate proteins do so by mimicking RNA or DNA targets of the regulated protein, as in the case of the 6S RNA in *E. coli* that inhibits RNA polymerase activity (125).

### Relevance of RNA-Based Regulation to Oligotrophs

Our primary argument here is that oligotrophs are generally reduced in transcriptional regulation compared to copiotrophs, while retaining other forms of regulation, including RNA-based regulation. This does not necessitate that oligotrophs have a greater abundance of RNA-based regulators on a per-kilobase basis, although this is a distinct possibility as discussed below. Rather, at the very least, the general lack of transcriptional regulation in oligotrophs means that other forms of regulation take on added importance in these cell types (24, 126). To our knowledge, there has not been any direct comparison of

the prevalence of RNA-based regulation between aquatic oligotrophs and copiotrophs, which offers an interesting further area of research. However, there are two pieces of evidence that suggest that marine oligotrophs may have larger numbers of RNA regulators than copiotrophs, although this will require further work.

The first piece of evidence comes from *Prochlorococcus*, where an impressively large number of RNA-based regulators has been reported (126), with over 73% of gene expression being linked to RNA regulators over a diel cycle (56). In addition, one of the two glutamine riboswitches (glutamine type 1) found in *Prochlorococcus* is unique in that it controls the expression of four genes, in contrast to the one gene generally regulated by glutamine type 1 riboswitches in other, more copiotrophic marine phytoplankton, hinting at an expanded role of this and, potentially, other riboswitches in *Prochlorococcus* (22). Finally, a high proportion (44%) of the primary transcriptome of two strains of *Prochlorococcus*, MED4 and MIT9313, are devoted to *cis*-sRNAs, much more than any other type of transcript, with *trans*-sRNA only comprising 5 to 9% of the transcriptome (127). Other, nonoligotrophic phytoplankton have much lower proportions of *cis*-sRNA (15 to 30%) (128).

The second piece of evidence comes from the vast amount of RNA molecules measured in marine metatranscriptomic studies from oligotrophic waters that are involved in regulation. Early studies of metatranscriptomic data found that riboswitches in particular are common and diverse in the environment, both in the oligotrophic open ocean and soil samples (129). At the Hawaii Oceanic Time Series (HOT), many measured transcripts (~16%) were small RNA molecules, many of which were either known to be regulatory or were putatively involved in regulation (130, 131). At multiple other oligotrophic, open ocean sites, a large proportion (~19%) of measured transcripts were found to be unannotated, >75-bp RNA transcripts, most likely regulatory RNA (132). In addition, when some of the most abundant transcripts measured at various sites in the open ocean were examined closely, they were found to be regulatory RNAs such as riboswitches (133).

### Examples of Riboswitches in Oligotrophs

SAR11 cells are known to have riboswitches from a variety of riboswitch classes, some of which appear to be unique to SAR11 cells and are of unknown functions (134). One of the best-studied examples is a pair of glycine riboswitches in "*Ca.* Pelagibacter ubique" strain HTCC1062. These cells are conditionally auxotrophic for glycine and serine as a consequence of gene losses that appear to have been driven by selection for small genome size. They elegantly regulate glycine metabolism with riboswitches to avoid glycine starvation, while channeling excess glycine and glycine precursor molecules to energy metabolism (4, 135). When intracellular glycine is low, the two independent, *cis*-acting glycine riboswitches repress transcription/translation of GlcB (malate synthase) and the aminomethyltransferase GcvT. As intracellular glycine concentrations rise, GlcB is activated by its riboswitch, channeling excess glyoxylate (a glycine precursor) into the TCA cycle. Higher intracellular glycine concentrations activate the second riboswitch, causing transcription/translation GcvT, which cleaves glycine to produce ATP, $NH_3$, and $CO_2$ (136). This unusual configuration, as seen also in the absence of the $P_{II}$ regulated system for nitrogen uptake in SAR11, appears to replace a common, vital, and complex regulated system with a system composed of fewer genes.

Two other instances of riboswitches being involved in essential metabolic pathways in SAR11 have also been uncovered. The response of *Pelagibacter* cells to sulfur limitation is largely mediated by riboswitches (69). The four most upregulated genes by *Pelagibacter* cells under sulfur limitation are all located downstream from *S*-adenosylmethionine riboswitches, which inhibit the translation of mRNA into protein (69). One of the most upregulated genes, *ordL*, was found to be located downstream of a conserved motif suggestive of a novel riboswitch (69). The response of *Pelagibacter* cells to sulfur limitation involves re-allocating sulfur to methionine instead of increasing expression of organosulfur transporter proteins, as was found to be the case for nitrogen and phosphate limitation (69). This was postulated to be due to *Pelagibacter* cells being adapted to a marine environment where organosulfur compounds are rarely

limiting (69). In addition, riboswitches are also thought to be involved in vitamin $B_1$ synthesis in *Pelagibacter* cells (137). *Pelagibacter* cells require thiamine, a vitamin $B_1$ precursor, for growth; the putative transporter for thiamine in these cells, ThiV, is located next to a riboswitch that binds thiamine-diphosphate (ThPP; the active form of vitamin $B_1$) (137). The authors of that study theorized that, when ThPP is present in sufficient quantities in the cell, it binds the riboswitch and prevents the transcription and/or translation of ThiV, preventing excess production of thiamine transporters (137).

As in SAR11, oligotrophic cyanobacteria such as *Prochlorococcus* have multiple instances of riboswitches, many of which are related to vitamin $B_{12}$ or $B_1$ metabolism, in addition to two types of glutamine riboswitches, as mentioned above (138). The breadth of riboswitches in *Prochlorococcus* has recently been reviewed at length (22).

### Examples of Small Noncoding RNAs in Oligotrophs

In oligotrophic cyanobacteria, there are a plethora of well-characterized sRNAs, many of which are involved in core processes such as photosynthesis and nitrogen metabolism (139). The recent review of *Prochlorococcus* regulation has in-depth discussion on sRNAs in *Prochlorococcus* (22). In general, *Prochlorococcus* tends to primarily utilize *cis*-sRNAs in regulation, as noted above. The sRNAs in *Prochlorococcus* have been shown to be involved in light adaptation, nitrogen limitation, and iron limitation, with a range of different sRNAs showing differential expression under different light regimes (126), nitrogen limitation (140), and iron limitation (141). *cis*-sRNAs are also involved in phage infection by protecting a wide set of mRNAs from degradation by RNase E, which is upregulated during phage infection (142).

In heterotrophic oligotrophic bacteria, there are several instances of sRNAs playing an important regulatory role. In the genomes of SAR86 cells, genome regions containing carbon assimilation pathways were found to be heavily populated with putative sRNAs (143). In the oligotrophic *Sphingopyxis granuli* strain TFA, isolated from river sediment, more than 90 putative sRNAs have been identified, with the *trans*-sRNA *suhB* playing an important role in the regulation of the degradation of tetralin, an environmentally contaminating hydrocarbon (144).

## POSTTRANSLATIONAL REGULATION

At the posttranslational level, enzyme activity can be modulated through either covalent modifications (often called posttranslational modifications [PTMs]) or allosteric regulation. This allows for rapid (seconds to minutes) responses to environmental stimuli that, in many cases, are reversible and spare the cell from the machinery needed for a transcriptional response (145). Posttranslational regulation has similar advantages to RNA-based regulation, with the added benefit of a potentially shorter time scale response, since translation is usually the slowest part of gene expression (146).

### Introduction to Covalent and Allosteric Posttranslational Regulation

Covalent posttranslational regulation (PTMs) involves the modification of proteins via the addition or removal of certain groups (e.g., phosphate, acetyl, methyl, etc.) or other modifications to amino acid residues (e.g., disulfide-bond formation or cleavage) (147). These modifications result in changes to enzyme function, the formation of protein complexes, etc. (148). There are numerous different types of PTMs, with acetylation and phosphorylation being two of the most widespread mechanisms (148, 149). The usage of PTMs as a regulatory mechanism in bacterial cells is only beginning to be understood but appears to be broadly used across bacterial taxa in diverse processes such as metabolism, protein synthesis, cell cycle, etc. (147–150), although modifications happen at a lower rate in bacteria than eukaryotes and at low stoichiometric levels, making detection challenging (147). In addition, different enrichment steps are needed for measuring different types of modifications, making it challenging to encompass all PTMs in one study. It was long thought that PTMs were only used in eukaryotes, since bacteria were too "simple" to use PTMs (151). Thus, it is only in recent

years that global studies of PTMs in a time-resolved manner in response to environmental stimuli have been conducted (150).

Allosteric posttranslational regulation involves the binding of a regulatory molecule to an enzyme at a noncompetitive (or allosteric) site, changing the conformation of the enzyme and thus its activity (152). Allosteric regulation differs from PTMs in that allosteric regulation is generally reversible, while PTMs are generally not reversible. Also, allosteric regulation generally does not require an effector enzyme to carry out the posttranslational regulation, as PTMs do. Similar to PTMs, allosteric regulation of enzyme activity has been understudied to date on a broad scale (153), although it is likely to be widespread (153, 154).

**Relevance of Posttranslational Regulation to Oligotrophs**

In theory, as with RNA-based regulation, posttranslational regulation might be especially relevant for oligotrophs, especially given the ability to modulate enzyme activity quickly, since translation generally is the longest part of gene expression, as noted before. However, as noted with RNA-based regulation, this does not necessitate that oligotrophs have a greater abundance of posttranslational regulators than copiotrophs, although this is possible. Between the two types of posttranslational regulation, allosteric is the most likely to be enriched in oligotrophs, since PTMs come with the added cost of needing an enzyme to carry out the PTM, which takes up genomic space, as well as the energy cost necessary to carry out the PTM. In addition, the most common type of PTM, phosphorylation, requires the use of phosphate, which is a limiting nutrient in the oligotrophic ocean. Allosteric regulation does not take up any genomic space, does not require energy input, and is readily reversible. Thus, if oligotrophs do have an enrichment in posttranslational regulation compared to copiotrophs, we would predict that allosteric regulation would be the most likely mechanism.

However, the hypothesis that oligotrophs have a greater reliance on allosteric regulation than copiotrophs will be challenging to fully examine on a whole-cell basis (sometimes called the "allosterome"). In fact, there are very few studies of the allosterome of a bacterial species, and the few that have been conducted are not comprehensive of all metabolic pathways in the cell (154–156). The challenges behind studying the allosteromes of cells, especially oligotrophs, are several: (i) the number of potential regulatory interactions in a cell is vast, given the large number of active enzymes in a cell and the large diversity of potentially regulatory small molecules in cells (variable by cell type and growth status [157]); (ii) allosteric regulation usually involves weak interactions, which are difficult to measure (156); and (iii) past large-scale computational studies identifying whole-cell allosteric interactions rely on decades of metabolic studies, something that is not currently available for most oligotrophs (155). Most likely, a whole-cell allosterome study comparing an oligotrophic and copiotrophic species is not plausible in the near future. Instead, as a start, focusing on one or more well-studied metabolic pathways and characterizing the allosteric interactions present in a model marine oligotroph and copiotroph would be a more achievable start, using some of the techniques already employed in model organisms (154–156).

We used our previously discussed functional comparison of marine oligotrophs and copiotrophs (Fig. 2) to address the question of whether genes involved in PTM are more common in oligotrophs than copiotrophs, with our prediction being that oligotrophs would have similar numbers of PTM genes as copiotrophs. We separated genes for known PTMs (acetylation, phosphorylation [kinases], ubiquitination, methylation, glycosylation, adenylation, and peptidases) into a separate category (O:PTM) from other category O (translation) genes. O:PTM genes were found to be at similar genomic proportions in both lifestyle categories, while the remaining category O genes were significantly enriched in oligotrophs (Fig. 2C). This could indicate that regulation via these known PTMs is used at similar rates within both copiotrophs and oligotrophs, in contrast to transcriptional regulation. One difficulty with this approach is that the PTMs used in this analysis have all been discovered and studied almost exclusively in copiotrophs. Further studies are needed to assess the relative importance of regulation

at the PTM level in oligotrophs compared to copiotrophs, both at the physiological and the genomic level.

### Examples of Posttranslational Regulation in Oligotrophs

Reported instances of posttranslational regulation in oligotrophs are rare, but a few have been reported. Several oligotrophic species from the *Sphingomonadales* order were found to have numerous instances of PTMs, both under normal growth conditions and in response to starvation, but the identity of the proteins modified and the nature of the modifications are unclear, as these experiments were conducted using two-dimensional electrophoresis methods (158–160). In oligotrophic *Nitrosopumilus maritimus* SCM1, 6% of the detected proteins were found to be modified by N-terminal acetylation under normal growth (72), which is a lower proportion than found in other archaea (~29%) (161). A recent study of L-alanine metabolism in SAR11 also found evidence for posttranslational regulation of the L-alanine transporter, perhaps via allosteric regulation, although this was not experimentally confirmed (76).

## KINETIC REGULATION

All of the types of regulation discussed so far have been part of hierarchical regulation, which modulate protein abundance/activity via the abundance of transcripts or enzymes. Kinetic regulation occurs outside of hierarchical regulation, where enzyme activities are modulated by metabolite-enzyme interactions, completely separate from the abundances of transcripts or enzymes (162). Thus, kinetic regulation is instantaneous and does not require extra genomic space or energy costs.

### Introduction to Kinetic Regulation

Kinetic regulation relies on different enzymes having different affinities for the same metabolites. Thus, at low levels of a given metabolite, it might be processed by a high-affinity enzyme, which sends it through one metabolic pathway. At higher concentrations, however, the same metabolite might be processed by an enzyme with lower affinity for the metabolite, but which has a higher activity, resulting in the metabolite being shunted down a different metabolic pathway. This form of regulation is widespread in cells and coordinates the flux of nutrients through cellular metabolism with hierarchical regulation, making it challenging to dissect on a case-by-case basis. Thus, many studies of kinetic regulation have been carried out in model organisms for central pathways on a large scale using modeling approaches. Examples include whole-cell modeling and use of $^{13}C$ flux analysis in *E. coli*, which found that kinetic regulation occurs at the level of individual intracellular metabolite concentrations, as well as a whole-cell coordination of metabolite uptake and growth rate (162–164).

### Relevance of Kinetic Regulation to Oligotrophs and an Example

As noted previously, forms of regulation that do not require excess genomic space or extra energetic/nutrient costs and are able to respond quickly are likely the most useful to aquatic oligotrophs. Since all of these are true of kinetic regulation, we would predict that kinetic regulation would be especially prevalent in oligotrophs. This was also the prediction of Button, who predicted that oligotrophs, due to the relatively constant nutrient concentrations that they are exposed to, might emphasize kinetic control more than hierarchical regulation (26). This hypothesis, however, will be challenging to study on a global scale, as noted with allosteric regulation, given the difficulties posed with studying kinetic regulation (noted above). However, the one reported instance of kinetic regulation in an aquatic oligotroph (regulation of dimethylsulfoniopropionate, DMSP, and metabolism in SAR11), provides support for our hypothesis that kinetic regulation is more prevalent in oligotrophs than copiotrophs. This regulation is illustrated in Fig. 5 in comparison to the regulation of DMSP metabolism in many copiotrophic bacteria.

DMSP is primarily metabolized in marine bacteria through either a cleavage pathway, which results in the gas dimethylsulfide (DMS) being released, or the demethylation

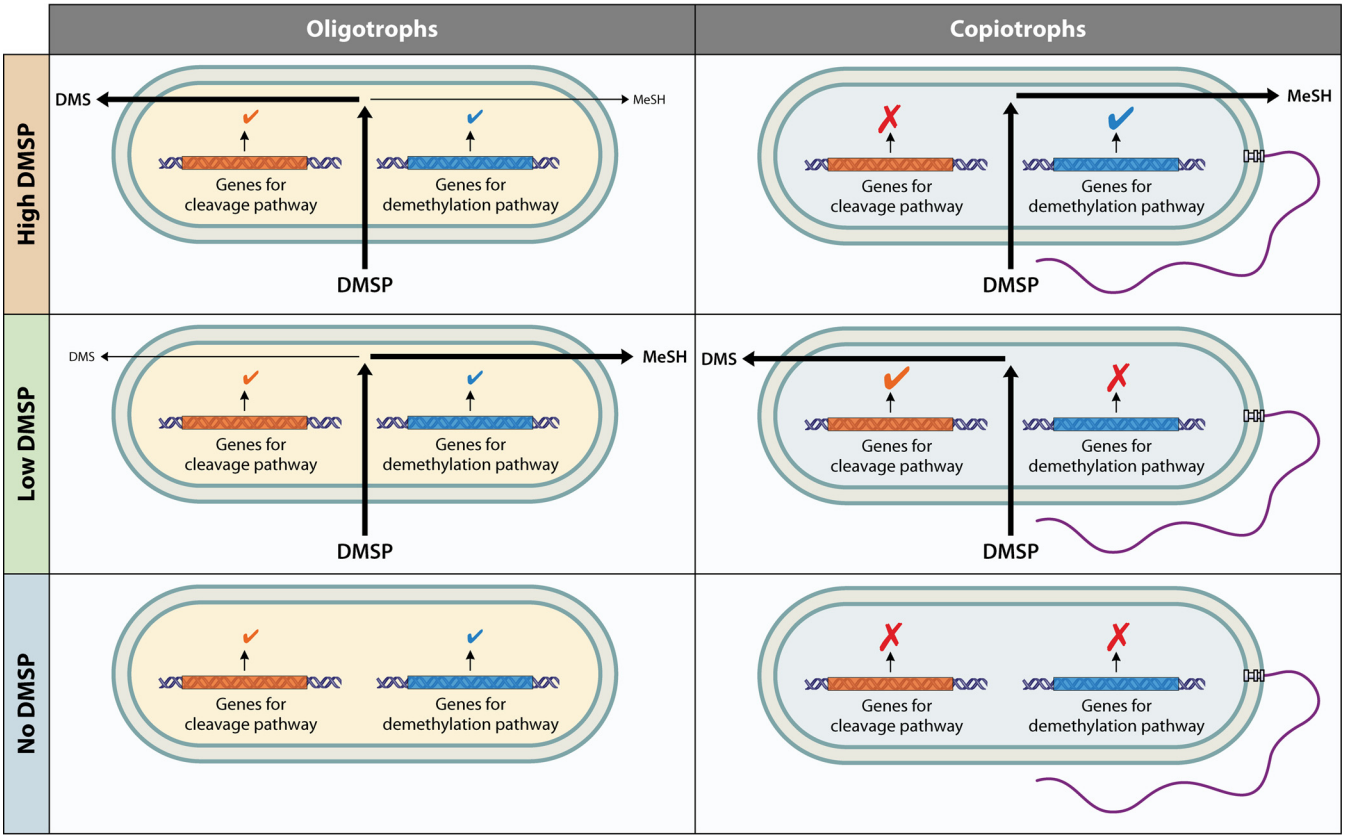


**FIG 5** Illustration of the differences between a common copiotrophic strategy of regulating dimethylsulfoniopropionate (DMSP) metabolism and the kinetic regulation of DMSP metabolism found in SAR11 cells. DMS, dimethyl sulfide; MeSH, methanethiol.

pathway, which results in the incorporation of the sulfur (S) from DMSP into biomass and release of methanethiol gas (73). In copiotrophic bacteria such as *Roseobacter* species, either DMSP itself or its catabolic products act as inducers of the DMSP lyase or demethylation genes (73). In the SAR11 strain "*Ca.* Pelagibacter ubique" HTCC1062, DMSP metabolism is under kinetic control instead of being transcriptionally regulated. No transcriptional response was observed in HTCC1062 cells in response to the addition of DMSP, and metabolism of DMSP did not increase upon preconditioning with DMSP (74). Instead, both the demethylation (mediated by DmdA) and cleavage (mediated by DddK) pathways are operational and constitutively expressed, resulting in the two pathways competing for metabolism of DMSP (74). However, DmdA has a higher affinity for DMSP than DddK, resulting in DMSP metabolism primarily being shunted into absorption of S from DMSP until cellular demands for S have been met, when increasing amounts of DMSP are shunted to the cleavage pathway for release as DMS (74). It seems that one of the adaptations of SAR11 to a life of oligotrophy is the replacement of transcriptional regulation of DMSP metabolism with kinetic regulation, reducing the amount of genomic coding and proteome complexity needed and increasing the speed of response.

## WHY DO OLIGOTROPHS USE LESS TRANSCRIPTIONAL REGULATION?

One of the primary questions that has yet to be definitively answered is why there is an evolutionary trend in oligotrophs to less transcriptional regulation; below, we propose three potential factors that may have played a role in this evolutionary trend, illustrated in Fig. 6.

One viable explanation is that transcriptional regulation has diminished fitness value in many of the niches found in nutrient-limited environments because of the elemental and energetic costs of transcriptional regulation. Cellular resources required for

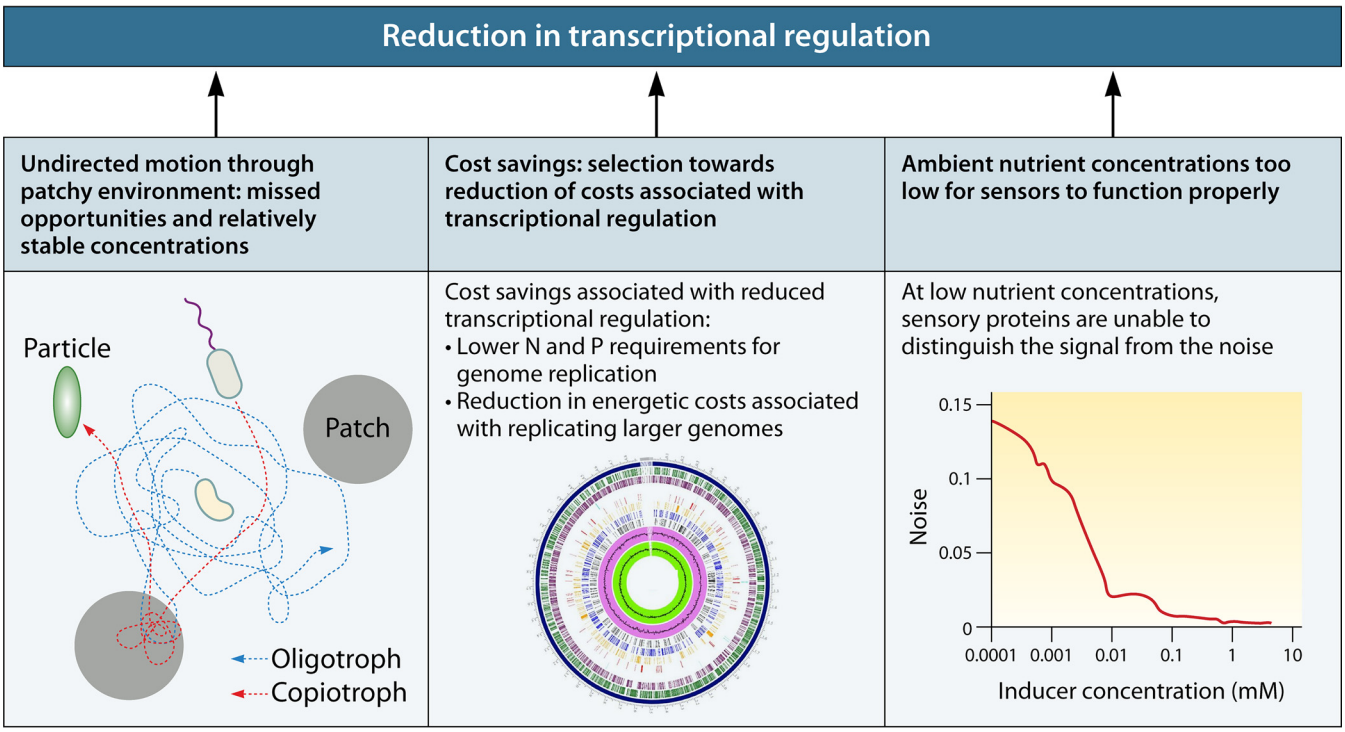


**FIG 6** Illustration of three potential factors that may have played a role in driving aquatic oligotrophs to a reduction in transcriptional regulation. See the text for a full discussion.

transcriptional regulation include the costs of coding sequences and transcribing and translating regulatory proteins (165). Most oligotrophs have streamlined genomes, with reduced intragenic regions, few pseudogenes and paralogs, an enrichment in nucleotides and amino acids with lower nitrogen content, and few regulatory genes (24). These adaptations are thought to be a strategy to reduce the cellular requirement for essential nutrients such as nitrogen and phosphorous that are limiting in oligotrophic environments (24). The reduction in genome complexity that accompanies the loss of transcriptional regulatory systems would, in theory, result in cells that need fewer macronutrients and less energy to reproduce (166, 167). However, it is an open question of whether the loss of transcriptional regulatory systems results in a large enough conservation of macronutrients and energy to explain the absence of transcriptional regulation in oligotrophs.

Another potential explanation for diminished regulation in oligotrophs lies in the nonmotile lifestyle of many oligotrophic cells. Cells in the ocean inhabit a patchy environment where they encounter ephemeral patches of high nutrient concentrations, interspersed within larger regions of low nutrient concentrations (168). Cells with transcriptional regulation must be able to turn on the genes needed to metabolize nutrients either before the patch dissipates, which can happen within 10 min of the patch forming (169, 170), or before they leave the nutrient patch. This is perhaps one reason why copiotrophs tend to be motile (14, 52), which allows them to locate and occupy nutrient patches and attach to particles, providing enough time to switch on metabolic enzymes. Oligotrophs, on the other hand, are generally nonmotile and/or nonchemotactic (Fig. 2) (14), possibly because their small cell size renders motility unfeasible due to Brownian effects, or because motility, like all genome complexity, seems to lose some of its fitness value in niches where resources are expensive (171, 172). Thus, nonmotile oligotrophs have no means to direct their drift through the water, resulting in a reduced amount of time spent in nutrient patches and an absence of time spent attached to particles (76, 169). If nonmotile oligotrophs relied on transcriptional regulation to turn on the necessary metabolic enzymes for nutrients

encountered in patches, the time required for these systems to initiate would preclude them from being able to make full use of the nutrients they encounter, as they would exit the patch before their metabolic systems are fully activated (76). The constitutive expression of most genes in oligotrophs, especially those related to carbon metabolism, means that the oligotrophs are able to instantly utilize whatever nutrients they encounter in a patch before they exit it, which reduces the cost of missed opportunities that nonmotile oligotrophs would otherwise incur.

On the other hand, the strategy of constitutively expressing genes for carbon metabolism incurs the costs of synthesizing proteins that have no use when the corresponding nutrient is absent. This strategy would not make much sense for a heterotrophic cell in a niche where the composition of the nutrient field varied qualitatively on time scales much larger than transcriptional response times. However, this strategy would make sense for cells that specialize in harvesting organic compounds that are not special, i.e., the small molecules and polymers common to all cell types. Enzyme multifunctionality has been reported in streamlined oligotrophs, such as SAR11 (173–175), which in principle broadens substrate range at no regulatory cost and potentially could be a factor offsetting the costs of deregulation strategies. This may be why heterotrophic oligotrophs are especially lacking in transcriptional regulation of carbon oxidation functions: there is a much wider diversity of carbon compounds available than inorganic nutrients. This strategy of constitutively expressing metabolic genes has been observed previously in *E. coli* cells that become adapted to a fluctuating environment as a memory mechanism or adaptive plasticity and has also been observed in thermal priming of dinoflagellates (39, 176). But instead of a short-term, epigenetic plasticity, oligotrophs seem to have undergone genomic adaptations to deal with the consistently low-nutrient, fluctuating ocean environment they experience.

Another potential reason for the lack of transcriptional regulation in oligotrophs revolves around the low ambient nutrient concentrations in the open ocean. If fluctuations in ambient nutrient concentrations tended to be below the threshold concentrations necessary to turn on regulatory systems, then, even if there was a potential advantage, regulation would not be effective. The threshold concentration above which motility is induced in marine bacteria has been found to be 0.001% (wt/vol) tryptic soy broth (177). A similar result was found in *E. coli*, where chemotaxis was not induced under a threshold concentration of a variety of compounds, and was only induced upon increases in nutrient concentrations that followed the Weber law (178). In marine diatoms, chemotaxis toward silicate particles was only induced when the silicate concentration was above a certain threshold (179). It may be that motility, with its large energy expenditure, is more tightly controlled based on nutrient concentrations than metabolic enzymes to diminish the potential for large costs to cells without benefits. This would argue for a second option, that cells can sense even low concentrations of nutrients, but do not expend energy to acquire them because of the risks (180).

The discussion above highlights some of the complexities in understanding why selection has favored different regulatory strategies depending on the pathway, the organism, and the niche. While some trends, for example the lesser amount of transcriptional regulation in oligotrophs, are clear, there are only a few examples from the cell biology of oligotrophs to illustrate other key concepts, and therefore our understanding of these issues will have to await investigations that find ways to probe these questions across a larger selection of different cell types.

## IMPLICATIONS OF THE OLIGOTROPHIC REGULATORY STRATEGY AND FUTURE PROSPECTS

Much of this review has focused on marine oligotrophs, since the majority of experimental studies of regulation in oligotrophs have been carried out in marine oligotrophs. Freshwater oligotrophs with streamlined features have been reported, as with the freshwater clade of SAR11 (181), but regulation in these organisms has been less studied. We would expect to see similar patterns of regulation in freshwater oligotrophs as well,

although this requires further confirmation. Similarly, in soil, it was long thought that large genomes were the norm, until the discovery of a highly abundant soil microbe with hallmarks of a streamlined genome (182). The adaptive pressures of soil environments are very different from aquatic environments, so it remains to be seen whether the same patterns of adaptation and genome evolution reported in marine oligotrophs will be repeated in soil oligotrophs. However, a previously described study that examined functional enrichment by lifestyle category across all environments (animal-associated, soil, marine, etc.) found a very strong depletion of genes involved in transcriptional regulation in oligotrophs (27). Thus, we predict that the pattern of regulation described here is generalizable to all oligotrophs across all environments. Nonetheless, it is clear that oligotrophs use transcriptional regulation when it is selectively advantageous, as described previously, and that different oligotrophs have different nuances of this general regulatory strategy.

In addition, much of our review has focused on two oligotrophic groups, SAR11 and *Prochlorococcus*, since most of the available information about regulation in aquatic oligotrophs has come from studies of these important model organisms, which are the most abundant microbial lineages in aquatic environments worldwide. As noted in the introduction, however, the oligotrophy/copiotrophy concept idealizes a spectrum of ecological strategies, so we would not predict that aquatic microbes are strictly dichotomous with respect to the alternate strategies we describe. Rather, the study of SAR11 and *Prochlorococcus*, which are the most successful oligotrophs in the world, offers broadly generalizable conclusions about the oligotrophic lifestyle that will likely vary based on a given oligotroph's niche.

One intriguing consequence of reduced transcriptional regulation in marine oligotrophs is that they may have less control over influxes of nutrients and growth rate, which is not a problem as long as their environment remains oligotrophic but could lead to metabolite imbalances in artificial environments. Increases in intracellular metabolites to toxic levels has been proposed as a cause of growth inhibition in oligotrophs exposed to high nutrient concentrations (175, 183). On the other hand, several instances have been reported in which very high intracellular accumulations of transported metabolites are metabolized later, providing these cells a means of exploiting transient nutrient surfeits. This "excess uptake" strategy has been observed in bulk marine microbial communities for glycine betaine (184), for DMSP in cultured SAR11 (74), and has been proposed to cause increases in polyamine storage and cell size, presumably from changes in turgor pressure, in SAR11 cells exposed to high polyamine levels (175).

The expansion of microbial diversity by metagenomics has revealed many new uncultivated lineages, including major new groups of abundant organisms that have highly reduced genomes such as the *Patescibacteria* and DPANN superphyla. Interestingly, although evidence about regulatory strategies in these two large groups is minimal, the genomic evidence available for *Patescibacteria* indicates many hallmarks of the oligotrophic strategy described above, with severe reductions in transcriptional regulatory proteins, two-component systems, and chemotaxis having been reported (185). In addition, the majority of reported *Patescibacteria* and DPANN genomes contain fewer $\sigma$-factors than expected per genome size (mean $\sigma$-factors/kb of 0.0066 and 0.043 for *Patescibacteria* and DPANN, respectively; one would expect closer to 0.01 for these genome sizes) based on a previously reported model fit for $\sigma$-factors and genome size (24, 186). In a number of cases, it has been established that *Patescibacteria* and DPANN superphyla are involved in symbioses (187, 188), providing a ready explanation for reductive genome evolution.

The evidence reviewed above indicates a dichotomy in paths to microbial success, wherein one path leads to versatile cells that can exploit environmental fluctuations, and incidentally grow well in labs, whereas the other path, seemingly more common, leads to success when relative stability in nutrient production and sustained competition combine to hold nutrient fluctuations in narrow ranges. Generally, when microbiologists observe very small genomes in nature, they suspect either mutualistic or parasitic symbioses. The patterns of gene regulation we report suggest an alternate explanation: if

simplicity/efficiency and complexity/versatility represent polar strategies for success, is it possible that some cells might be prospering in nature that are even more simple than the global oligotrophs that have become model organisms? By recognizing that the imprint of oligotrophy on regulation is not simply diminished transcriptional regulation but instead fine network tuning by selection to achieve success in a different niche, we might be able to recognize more extreme examples of reductive evolution that are due to environmental adaptation rather than coevolution.

## CONCLUSIONS

Like many aspects of biology, regulatory strategies of microorganisms are more complicated than was originally thought. With the increased awareness of the importance of oligotrophic microbes to environmental processes, more and more oligotrophs are being brought into culture, where the nuances of their regulatory strategies can be studied more closely. The realization that many abundant oligotrophs have reduced transcriptional regulation and constitutively express most of their genes for carbon oxidation has led to the testable hypothesis that oligotrophs instead rely primarily on posttranscriptional regulation. As has been noted above, many types of posttranscriptional regulation have only begun to be explored, especially in oligotrophs. For example, the breadth of potential riboswitches in microbes is still being uncovered and offers a promising avenue for further discoveries of posttranscriptional regulation. Both posttranslational and metabolic regulation are other vast, underexplored fields, especially in oligotrophs. We hope this review has communicated to readers that there are broad trends in regulation that distinguish oligotrophs from copiotrophs, even if as yet we do not fully understand the selective pressures that drive these trends or the integration of different types of regulation in relation to these two very different trophic strategies.

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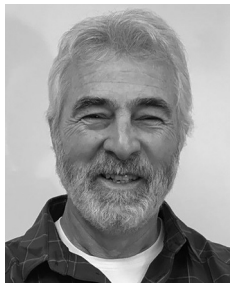

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