ANNUAL REVIEWS

Annual Review of Microbiology

Hostile Takeover: How Viruses Reprogram Prokaryotic Metabolism

Tyler B. Jacobson,[1,2,3] Melanie M. Callaghan,[1,3] and Daniel Amador-Noguez[1,2,3]

[1]Department of Bacteriology, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA; email: tbjacobson@wisc.edu, callaghan2@wisc.edu, amadornoguez@wisc.edu

[2]Great Lakes Bioenergy Research Center, University of Wisconsin–Madison, Madison, Wisconsin 53726, USA

[3]Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

Annu. Rev. Microbiol. 2021. 75:515–39

First published as a Review in Advance on August 4, 2021

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-060621-043448

Keywords

metabolism, archaeal viruses, bacteriophages, virus physiological phenomena, metabolic networks and pathways, viral infections, auxiliary metabolic genes, AMGs

Abstract

To reproduce, prokaryotic viruses must hijack the cellular machinery of their hosts and redirect it toward the production of viral particles. While takeover of the host replication and protein synthesis apparatus has long been considered an essential feature of infection, recent studies indicate that extensive reprogramming of host primary metabolism is a widespread phenomenon among prokaryotic viruses that is required to fulfill the biosynthetic needs of virion production. In this review we provide an overview of the most significant recent findings regarding virus-induced reprogramming of prokaryotic metabolism and suggest how quantitative systems biology approaches may be used to provide a holistic understanding of metabolic remodeling during lytic viral infection.

Contents

1. INTRODUCTION

Prokaryotic metabolic processes are tightly regulated to achieve a precise balance between nutrient assimilation, energy generation, and biosynthetic activity that enables efficient production and assembly of cellular components at the correct proportions. Furthermore, to ensure survival and reproduction in dynamic natural environments, prokaryotic organisms must be capable of quickly adjusting their metabolism in response to changes in nutrient availability and other environmental variables. Prokaryotic viruses hijack this finely tuned system for their own reproduction. In the typical viral life cycle, a viral particle adsorbs to a potential host and injects its genome. Within the host, viral gene expression redirects host biosynthesis to the production of new virions that escape the host cell by budding or lysis and infect new hosts. The infected host cell that has been repurposed into a virion factory is termed a virocell, a biological entity with a metabolism distinct from that of uninfected host cells (51).

Prokaryotic virus: virus infecting either archaea or bacteria

De novo nucleotide synthesis: synthesis of nucleotides from newly produced precursors; does not include recycling of existing nucleic acid, nucleoside, or nucleobase components

Virions are composed of a DNA or RNA genome encapsulated by a protein capsid commonly adorned with additional proteinaceous structures, making them enriched for nucleic acids and proteins relative to their prokaryotic hosts. Thus, virion production is an energetically demanding process that requires substantial supplies of both nucleotides and amino acids (101, 168). While a portion of these biosynthetic precursors can be obtained from existing intracellular pools and/or from the degradation of host cellular components, these sources may not be enough to satisfy the exacting demands of virion production, and de novo synthesis of these precursors may be necessary. De novo nucleotide synthesis requires ribose phosphate, one-carbon units, ammonia, phosphate, and specific amino acids; amino acid synthesis requires central carbon intermediates and a nitrogen donor. Both nucleotide and amino acid synthesis pathways, as well as downstream viral genome

replication and protein synthesis, utilize large amounts of energy and reducing power (101). It would therefore be highly advantageous for viruses to commandeer host biosynthetic capacity during infection to ensure an adequate supply of energy, reducing power, and biosynthetic precursors for the production of virion components. Such a reconfiguration of cellular metabolism requires coordinated control of diverse metabolic processes, and viruses may employ multiple strategies to accomplish this, including (*a*) manipulating expression of host metabolic genes, (*b*) using virally encoded metabolic enzymes, and (*c*) using virally encoded regulatory elements that modulate activity of host enzymes. Using these strategies, viruses can increase metabolic flux through desired pathways, maintain or enhance activity of energy- and reducing power–generating processes, and downregulate competing pathways or cellular processes that do not contribute to the synthesis of virion components. Not surprisingly, the extent to which a specific virus will use these strategies varies widely, and a virus can still be successful while employing only a subset of them (35).

Metabolic flux analysis: technique used to measure in vivo metabolic fluxes, often incorporating isotope-tracer data

Recent findings support the paradigm outlined above. Most notably, the advent of advanced sequencing technologies, including metagenomics, has produced a large catalog of virus-encoded genes with putative metabolic functions, ranging from genes encoding metabolic enzymes to those encoding regulatory proteins. However, the in vivo role of viral effectors in host metabolism is only beginning to be unraveled.

In this review, we discuss some of the most significant recent findings regarding virus-induced reprogramming of microbial metabolism and suggest how quantitative systems biology approaches such as isotope-based metabolic flux analysis and computational modeling may be used to provide a holistic description of metabolic remodeling during viral infection.

2. VIRAL REMODELING OF CYANOBACTERIAL PHOTOSYNTHESIS

Marine cyanobacteria use oxygenic photosynthesis to generate the chemical energy (ATP) and reducing power (NADPH) needed for autotrophic growth and carbon fixation via the Calvin cycle (45, 48). During this process, light energy drives the transfer of electrons from water to NADPH via photosystems II and I (PSII and PSI). This linear electron flow generates a proton motive force from which ATP synthase produces ATP. Light energy can also power a cyclic electron flow around PSI (i.e., electrons are recycled back to PSI instead of being used to reduce $NADP^+$) to generate additional proton motive force for ATP generation.

Viruses that infect marine cyanobacteria (i.e., cyanophages) are prevalent in oceanic environments and have received considerable attention in recent years. Cyanophages commonly encode homologs of host proteins distributed across every step of oxygenic photosynthesis, including electrons' passage through PSII and PSI reaction centers and other components of the electron transport chain, the Calvin cycle, the pentose phosphate pathway, light harvesting, and bilin metabolism. As we describe below, expression of these viral genes within the virocell is thought to result in a redirection of photosynthetic electron flow that increases ATP generation and a remodeling of carbon metabolism to increase production of ribose 5-phosphate (R5P) and NADPH regeneration. These metabolic modifications are thought to enhance viral nucleic acid synthesis and replication.

2.1. Remodeling of Photosynthetic Electron Flow by Cyanophages

Homologs of the *psbA* and *psbD* genes, which encode the labile D1 and D2 proteins that form the heterodimeric core of the PSII reaction center, are widely distributed among sequenced cyanophages that infect *Prochlorococcus* spp. or *Synechococcus* spp. (94, 104, 105, 107, 156, 158). The D1 and D2 core proteins are susceptible to photodamage and must be continuously replaced to maintain PSII activity (11, 141, 161). However, during viral infection the expression of host photosystem components drops, which compromises the repair and thus function of PSII

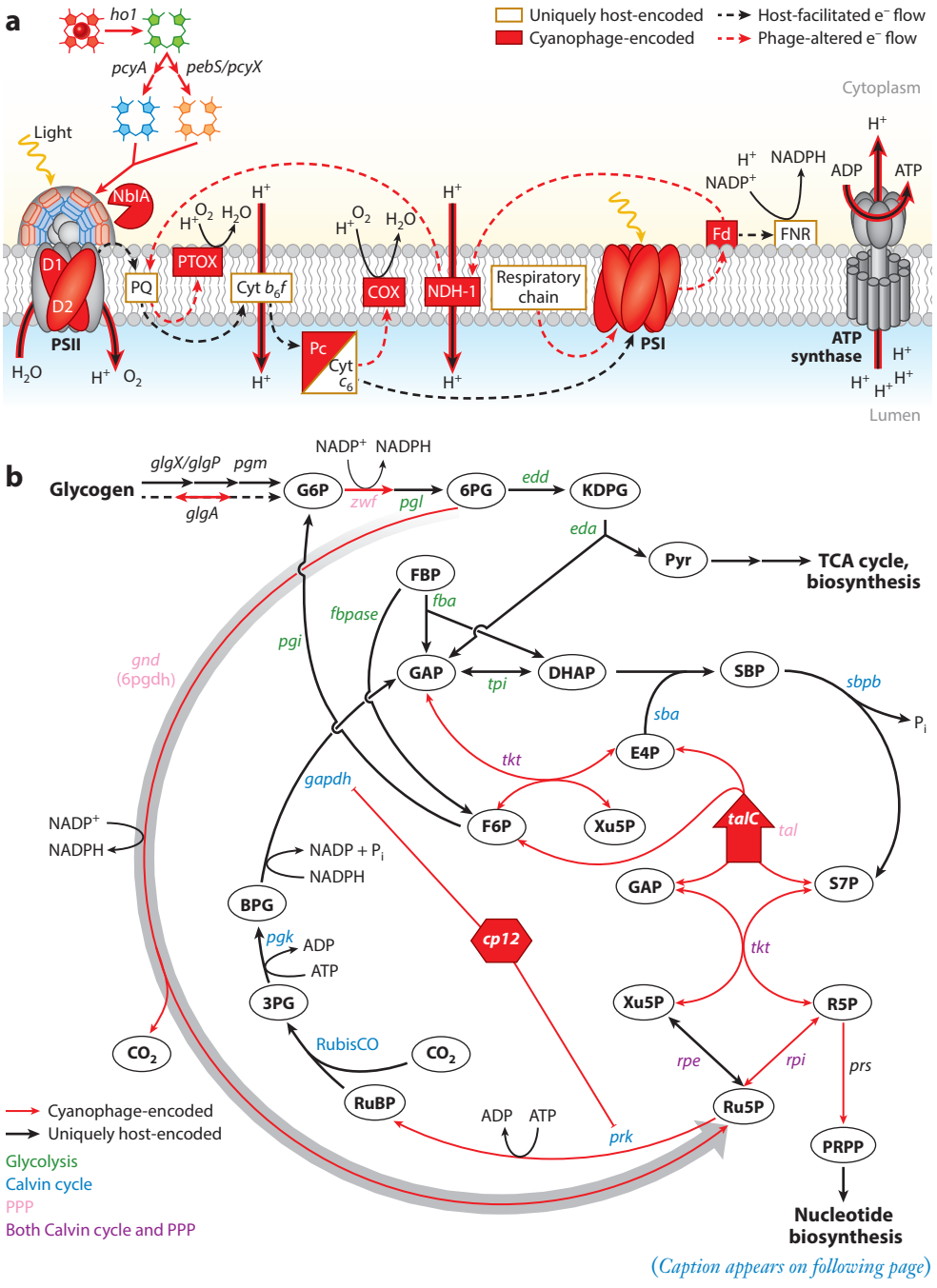
(92, 93). Since cyanophages are thought to rely on photosynthesis to generate energy and reducing power for replication (15, 30, 58, 92, 93), the expression of phage-encoded D1 and D2 may be critical to maintain or enhance PSII activity during infection (30, 38, 92–94, 156). In support of this hypothesis, it was observed that *Synechococcus* cells infected with a cyanophage (i.e., S-PM2) possessing *psbA* and *psbD* homologs maintained PSII reaction center activity better than uninfected cells under high-light conditions (135). The rate of D1 and D2 protein turnover during infection of *Synechococcus* with another cyanophage (i.e., S-SM1) also possessing *psbA* and *psbD* homologs increased in proportion to light intensity, even though host protein turnover decreased (168); this is consistent with phage *psbA* and *psbD* supporting maintenance of PSII function as host protein expression diminishes (52, 92, 93, 168).

Indicating a broader involvement in host photosynthesis beyond simple replacement of damaged PSII core proteins, marine phages have also been found to encode photosynthetic electron transport proteins. For example, a homolog of *petE*, encoding the soluble electron carrier plastocyanin (Pc), is commonly found in cyanophages (94, 108, 156, 157, 171). It has been hypothesized that phage-encoded Pc is capable of donating electrons to the terminal electron acceptor cytochrome terminal oxidase (COX) in the respiratory chain instead of passing them on to PSI (95, 136). By preventing the flow of electrons from PSII to PSI, and thereby bypassing NAD(P)H generation by ferredoxin:$NADP^+$ oxidoreductase (FNR), this would effectively create a light-powered cyclic electron flow around PSII capable of producing a proton gradient that can be used to generate ATP (**Figure 1*a***). In a similar vein, cyanophages encode a putative plastid-terminal oxidase (PTOX) (43, 108, 157, 171), which is hypothesized to receive electrons from plastoquinone (PQ) and donate them to oxygen (12). This would also prevent electron flow to PSI and result in a proton gradient–generating cyclic flow of electrons around PSII (**Figure 1*a***). Indeed, infection of *Prochlorococcus* with phage H-PM2 results in upregulation of host COX, PTOX, and PSII components while lowering expression of host FNR (162).

In addition to promoting cyclic photophosphorylation around PSII, some phages appear to also increase cyclic electron flow around PSI during infection. PSI gene cassettes containing up to seven PSI genes (*psaJF*, *psaC*, *psaA*, *psaB*, *psaK*, *psaE*, and *psaD*) are present in marine phages (52, 148). It was recently shown that infection of a *Prochlorococcus* host with a cyanophage encoding PSI, PSII, and NAD(P)H dehydrogenase (NDH-1) complex proteins resulted in enhanced cyclic electron flow around PSI, likely as a strategy to increase ATP availability within the virocell (52). Interestingly, PSII activity remained unchanged during infection, which suggests that a PSII-independent electron flow to PSI was somehow induced. NDH-1 is a proton pump that participates in cyclic electron flow around PSI by transferring electrons from ferredoxin (Fd) to the PQ pool, bypassing NAD(P)H generation by FNR. Cyanophage-encoded subunits of the NDH-1 complex are thought to be important for redirecting electrons away from the respiratory chain to PSI via the PQ pool (79, 95, 136, 149), and their expression during infection is thus expected to boost PSI cyclic photophosphorylation (**Figure 1*a***). Providing another potential mechanism to increase electron flow to PSI, the viral PSI PsaJF fusion protein is hypothesized to allow PSI to receive electrons from alternate donors, including cytochromes from the respiratory electron transport chain, in addition to the canonical donor Pc (136, 148). Finally, many cyanophages possess a homolog of *petF*, encoding the electron carrier Fd (94, 108, 156, 157). Cyanophage P-SSM2 *petF* is most similar to a class of cyanobacterial Fds that are known to interact with PSI, so its expression during infection may help enhance cyclic electron flow around PSI (20). Alternatively, since the same class of Fds also participate in electron transfer to enzymes involved in nutrient assimilation and biosynthetic pathways, such as nitrite reductase and heme oxygenase (discussed in Section 2.3), cyanophage Fd may redirect electrons away from the electron transport chain and toward nutrient assimilation or biosynthesis (discussed in Section 5.3) (20, 34, 86, 87).

2.2. Metabolism of Light-Harvesting Components

Cyanobacteria use antenna complexes to absorb light energy for photosynthesis. The primary antennae in *Synechococcus* are phycobilisomes, large complexes of proteins and light-absorbing



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Cyanophages alter photosynthetic electron transport and carbon metabolism in their cyanobacterial hosts. (*a*) Electron flow in virocells relies on both host-encoded and phage-encoded proteins, facilitating cyclic electron flow around PSI and PSII. Components that have been identified in phages are in red shapes; exclusively host-encoded components are in white. Dashed arrows indicate electron flow specifically, whereas solid arrows indicate reactions/metabolite transitions and proton pumping. Genes involved in phycobilisome pigment synthesis (*red*, heme; *green*, biliverdin; *blue*, phycocyanobilin; *orange*, phycoerythrobilin) have also been found in phages. (*b*) Cyanophages encode genes to alter carbon metabolism, directing flux toward Ru5P and nucleotide biosynthesis and potentially facilitating glycogen utilization. Phage-encoded CP12 (*red hexagon*) and TalC (*red arrow*) work together to direct carbon away from the Calvin cycle and into nucleotide synthesis precursors via the pentose phosphate pathway. While GlgA is known to participate in glycogen synthesis, we hypothesize that phage-encoded GlgA may be capable of acting in the reverse direction. Colors of gene names indicate metabolic pathways. Abbreviations: 3PG, 3-phosphoglycerate; 6PG, 6-phosphogluconate; 6pgdh, 6-phosphogluconate dehydrogenase; BPG, 1,3-bisphosphoglycerate; COX, cytochrome terminal oxidase; cyt, cytochrome; DHAP, dihydroxyacetone phosphate; E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; Fd, ferredoxin; FNR, ferredoxin:$NADP^+$ reductase; G6P, glucose 6-phosphate; GAP, glyceraldehyde phosphate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; NDH-1, NAD(P)H dehydrogenase; Pc, plastocyanin; P_i, inorganic phosphate; PPP, pentose phosphate pathway; PQ, plastoquinone; PRPP, phosphoribosyl pyrophosphate; PSI, photosystem I; PSII, photosystem II; PTOX, plastid-terminal oxidase; Pyr, pyruvate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; RubisCO, RuBP carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; S7P, sedoheptulose 7-phosphate; SBP, sedoheptulose 1,7-bisphosphate; TCA, tricarboxylic acid; Xu5P, xylulose 5-phosphate.

pigments (i.e., bilins), while antennae in *Prochlorococcus* are smaller complexes centered around chlorophyll a and b derivatives (61, 165, 166). Some cyanophages contain a variety of genes related to bilin metabolism, including *ho1*, *pcyA*, *pcyX*, and *pebS*, whose products convert heme to bilin used in phycobilisomes, and *cpeT*, which is homologous to a host lyase that attaches bilins to phycobilisome structural proteins, though phage *cpeT* apparently lacks this function (19, 34, 55, 86, 87, 157, 171) (**Figure 1*a***). The function of these genes during infection is unclear, as many are unique to phages of *Prochlorococcus*, which does not use phycobilisome-based antennae, and these phages do not encode any phycobilisome structural proteins (61). One possibility is that phage infection induces production of an antenna complex unique to the virocell that can incorporate bilins produced by phage-encoded enzymes. Alternatively, genes like *ho1*, whose product cleaves heme, may function in support of PSI activity by freeing iron from heme that can then be used by PSI reaction centers. If this is the role of *ho1*, we would expect to see enrichment in phages infecting clades of cyanobacteria known to inhabit iron-depleted waters, though phage encoding of further bilin synthesis enzymes (PcyA, PcyX, PebS) makes iron access unlikely to be the sole reason for phages to encode *ho1* (44).

Some phages might also induce degradation of antenna complexes. For example, freshwater cyanophages, such as Ma-LMM01 and PaV-LD (54, 180), and marine viral metagenomes (116) encode a homolog of NblA, which enhances phycobilisome degradation in cyanobacteria by recruiting proteases (54, 116, 118, 181). Heterologous expression of NblA in *Synechocystis* results in a spectroscopic shift associated with the loss of phycobilisome-associated pigments, suggesting that phage NblA also induces phycobilisome degradation (54). The phages known to encode NblA do not encode reaction center D1 or D2 homologs and are thus presumably less able to induce photosystem repair. NblA may thus play a compensatory role; by degrading phycobilisomes and reducing the amount of light energy absorbed, phage NblA may protect the photosynthetic apparatus from photodamage, helping the phage maintain photosynthetic energy generation during infection (54, 116, 118, 180, 181). Alternatively, phycobilisome degradation may release

nutrients, such as amino acids, that can then be used for phage replication (32, 33, 56, 93). Instead of degrading phycobilisomes to protect the host photosynthetic apparatus, many cyanophages encode high-light-inducible proteins (94, 107, 108, 156, 157), which protect the photosynthetic apparatus from damage due to oxidation or high-light conditions (80).

2.3. Remodeling of Carbon Metabolism by Cyanophages

Carbon fixation via the Calvin cycle is a major consumer of ATP and NADPH in phototrophic organisms. Many cyanophages encode a homolog of the regulatory protein CP12, which binds and inhibits two enzymes in the Calvin cycle: (*a*) phosphoribulokinase (Prk), which catalyzes the ATP-dependent phosphorylation of ribulose 5-phosphate to ribulose 1,5-bisphosphate, and (*b*) glyceraldehyde-3-phosphate dehydrogenase, which catalyzes the NADPH-dependent conversion of glycerate 1,3-bisphosphate to glyceraldehyde 3-phosphate (**Figure 1*b***). Thus, expression of CP12 during infection would be expected to stop or decrease activity of the Calvin cycle, preserving ATP and NADPH for virocell-specific processes. Indeed, it has been shown that cyanophages inhibit CO_2 fixation (137). Most cyanophages encoding CP12 also contain *talC*, which encodes an enzyme from the nonoxidative pentose phosphate pathway (PPP). TalC likely has a dual purpose within virocells: to help increase flux through the nonoxidative PPP to generate R5P for nucleotide synthesis and to compete with enzymes from the Calvin cycle for substrates, namely, sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate, helping further suppress carbon fixation (66, 136, 163).

Some cyanophages also have genes encoding enzymes from the oxidative PPP, including glucose 6-phosphate dehydrogenase (*zwf*) and 6-phosphogluconate dehydrogenase (*gnd*) (66, 92, 157, 163, 171). Although there is currently no experimental evidence regarding the role of cyanophage-encoded Zwf and Gnd, we expect that their expression during infection would increase flux through the oxidative PPP, thereby increasing ribulose 5-phosphate and NADPH production. The oxidative PPP is critical to restarting photosynthetic metabolism during the dark-to-light transition, and the carbon needed for this process (in the form of glucose 6-phosphate) comes from degradation of intracellular glycogen reserves; we thus expect that during phage infection, increased activity of the oxidative PPP is also fueled by glycogen reserves (37, 151, 152, 188). Indeed, this fits well with the known dependence of cyanophage adsorption and infection on the presence of light, which causes infection to coincide with high glycogen availability or photosynthetic potential to fuel virocell metabolism (73, 96, 119, 172). While, to our knowledge, there are no reports of cyanophages encoding glycogen breakdown enzymes, expression of cyanophage-encoded sigma factors has been shown to increase abundance of host transcripts of glycogen phosphorylase and debranching enzymes, *glgP* and *glgX*, suggesting that phages may mediate glycogen metabolism by modulating host gene expression (146). Interestingly, carbon metabolism in cyanophage-infected cells is reminiscent of metabolism during the dark phase of the uninfected cyanobacterial life cycle: decreased carbon fixation and increased catabolism of existing carbohydrate stores. Indeed, the most-upregulated genes during the dark cycle, *cp12*, *talC*, *zwf*, and *gnd*, are among the most abundant in cyanophages (188).

In summary, our current understanding suggests that cyanophage infection results in a drastic reorganization of both photosynthetic electron flow and carbon metabolism that involves (*a*) increased cyclic electron flow around PSII and PSI for ATP generation, potentially at the expense of NADPH generation; (*b*) shutdown of the Calvin cycle to conserve ATP and NADPH; and (*c*) mobilization of glycogen reserves and increased oxidative PPP activity for the generation of R5P and NADPH (**Figure 1*b***).

3. VIRAL MODULATION OF NONPHOTOSYNTHETIC ENERGY PRODUCTION

3.1. Ammonia and Sulfur Oxidation

Ammonia-oxidizing bacteria and ammonia-oxidizing archaea are chemolithoautotrophic organisms that obtain energy and reducing power from the oxidation of ammonia (NH_3) to nitrite (NO_2^-). The initial step in this process is carried out by ammonia monooxygenase in both ammonia-oxidizing bacteria and ammonia-oxidizing archaea. Electrons originating from ammonia oxidation are directed toward NDH-1 to produce NADH or toward a terminal oxidase to generate a proton gradient that drives ATP production (81, 154).

Sequences homologous to the AmoC subunit of ammonia monooxygenase are prevalent among viruses infecting marine ammonia-oxidizing archaea from the phylum *Thaumarchaeota*, which are abundant in marine ecosystems and play an important role in the global nitrogen cycle (2, 143). While the precise role of the AmoC subunit remains unclear (167), it is plausible that its expression helps maintain ammonia oxidation, and thus energy and reducing power generation, during infection (2, 143).

It has recently been shown that spindle-shaped viruses that infect marine ammonia-oxidizing *Thaumarchaeota* are able to maintain normal rates of ammonia oxidation for a period of multiple days after infection, even after host growth and replication have been shut down (77). Importantly, virus production was largely tied to the period in which ammonia oxidation was maintained, supporting the hypothesis that ATP and reducing power obtained from ammonia oxidation are directed toward and required for virus replication.

Sulfur-oxidizing bacteria obtain energy and reducing power from the oxidation of sulfur compounds. Marine viral metagenomes collected from surface and deep-sea waters have been found to contain sequences homologous to *soxYZ* or *rdsrA* and *rdsrC*, which catalyze the reversible oxidation of sulfur compounds, such as thiosulfate to sulfate or sulfur to sulfite (4, 143). Viral encoding of these enzymes may allow virocells to maintain sulfur oxidation–based energy generation when infecting sulfur bacteria (114).

In addition to phage encoding of ammonia- and sulfur-oxidation enzymes, analyses of viral metagenomes have found enrichment of genes encoding components of the formate dehydrogenase–O and formate dehydrogenase–N complexes (*fdoG* and *fdnI*, respectively). Both enzymes oxidize formate to CO_2, allowing formate to serve as an electron donor during respiration (6, 13, 42, 134).

3.2. Electron Transport Chain Restructuring in *Pseudomonas aeruginosa*

Similar to the case of virus-induced remodeling of photosynthetic electron transport chains in cyanobacteria and maintenance of ammonia oxidation in ammonia-oxidizing archaea, phages that infect the opportunistic pathogen *Pseudomonas aeruginosa* appear to modulate activity of the respiratory electron transport chain. However, unlike cyanophages and thaumarchaeal viruses, *P. aeruginosa* viruses do not encode homologs of electron transport chain components but instead induce changes in host expression of these genes (28, 84, 186).

For instance, *P. aeruginosa* infection with the LUZ19 phage is associated with increased transcription of genes encoding nitrite reductase (*nirS*), its associated cytochromes (*nirC* and *nirM*), and the dehydrogenase (*nirN*) responsible for producing the d_1 heme cofactor used by NirS. The concerted upregulation of these genes likely increases the use of nitrite as the final electron acceptor. In addition, transcription of the low-affinity, oxygen-dependent aa_3 cytochrome *c* oxidase increases, while transcription of a subunit of a high-affinity cytochrome *c* oxidase is downregulated.

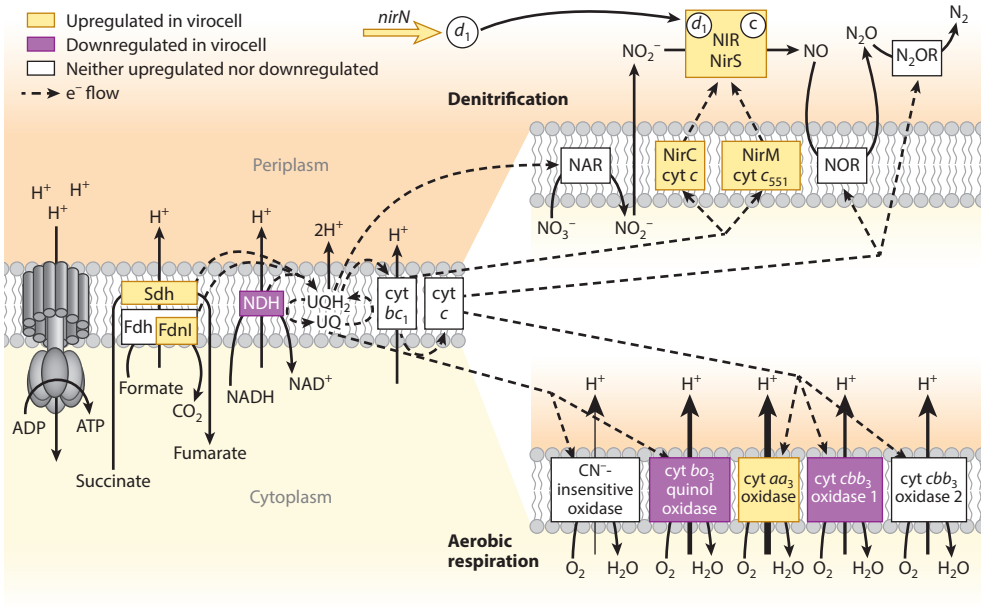


Figure 2

Pseudomonas aeruginosa virocells have altered electron transport chains. LUZ19 infection increases nitrite reductase expression and supports proton motive force generation while conserving NADH reducing power. Elements in yellow rectangles are upregulated during infection; those in purple rectangles are downregulated. Arrow thickness for pumped protons during aerobic respiration positively correlates to the ratio of protons per pair of electrons transferred to H_2O. Abbreviations: cyt, cytochrome; Fdh, formate dehydrogenase; FdnI, subunit of Fdh; N_2OR, nitrous oxide reductase; NAR, nitrate reductase; NDH, NADH dehydrogenase; NIR, nitrite reductase; NOR, nitric oxide reductase; Sdh, succinate dehydrogenase; UQ, ubiquinone.

This shift towards *aa*$_3$ cytochrome *c* oxidase utilization may enhance energy generation in infected cells since this oxidase translocates a larger number of protons per electron donated to oxygen. Interestingly, transcription of multiple components of NADH dehydrogenase decreases during infection, suggesting that the use of NADH as an electron donor in the respiratory chain is disrupted. This could help conserve reducing power for viral production but would reduce electron supply to the respiratory chain (84). As a potential compensatory mechanism, protein abundance of succinate dehydrogenase (Sdh) and transcription of *fdnI*, the nitrate-inducible formate dehydrogenase, both increase. This suggests that succinate and formate may replace NADH as the favored electron donor for respiration in infected cells (**Figure 2**). Finally, LUZ19 infection also results in increased transcription of malonate decarboxylase and a malonate transporter; this may prevent malonate from competitively inhibiting Sdh but may also indicate increased utilization of malonate as a carbon or energy source (100).

Modulating the expression of host electron transport chain components to increase energy generation or preserve reducing power may be a common strategy employed by prokaryotic viruses. Besides LUZ19, two other *P. aeruginosa* phages (28, 186), two *Bacteroidetes* phages (63, 65), and two phages of *Pseudoalteromonas* (64) have been observed to induce transcriptional changes in host genes encoding respiratory nitrate and nitrite reductases, succinate dehydrogenases, ferredoxins, cytochromes, cytochrome oxidases, NADH:quinone reductases, and ATP synthase subunits.

4. NUCLEOTIDE SYNTHESIS

Prokaryotic viruses have two potential sources of nucleotides for their genome replication: de novo synthesis or recycling of existing nucleotides through degradation of host DNA and RNA. Recycling of existing nucleotides is a well-established aspect of phage biology (31, 103, 106, 109, 110, 117, 155, 173), but more recently, phages have been found to encode components of de novo purine and pyrimidine synthesis pathways or to upregulate host nucleotide synthesis during infection.

4.1. Modulation of Nucleotide Synthesis by Phage-Encoded Enzymes

Homologs of the gene *prs* have been found in some *Salmonella*, *Aeromonas*, and marine gammaproteobacterial phages as well as in marine viral metagenomes. This gene encodes the enzyme ribose-phosphate pyrophosphokinase, which catalyzes the conversion of R5P to phosphoribosyl pyrophosphate, a requisite biosynthetic precursor for both purine and pyrimidine de novo synthesis (10, 66, 112, 121). A cyanophage-encoded *prs* protein has not been reported so far. Instead, some cyanophages encode carbamoyl phosphate synthetase and aspartate-carbamoyltransferase homologs, which catalyze the first steps in de novo pyrimidine biosynthesis (6, 75, 156, 157). Other homologs of de novo pyrimidine (*pyrE*) and purine (*purH*, *purL*, *purM*, *purN*) biosynthetic pathway genes have also been observed in cyanophages and marine viral genomes (26, 144, 156) (**Figure 3**). These findings suggest that cyanophages, as well as other viruses, actively strive to increase or maintain de novo nucleotide synthesis within the virocell. This aligns well with the previously discussed hypothesis that cyanophages remodel host carbon metabolism to increase R5P production for nucleotide synthesis.

Nucleotide synthesis requires one-carbon units provided by tetrahydrofolate (THF) cofactors. During purine synthesis, PurN and PurH spend N^{10}-formyl-THF, producing THF. Synthesis of dTMP by thymidylate synthase consumes 5,10-methylene-THF (5,10-MeTHF) and produces 7,8-dihydrofolate (DHF) (**Figure 3**). Marine and rumen viral metagenomes encode homologs of serine hydroxymethyltransferase (SHMT), which is an enzyme that regenerates 5,10-MeTHF from THF by taking a one-carbon unit from serine, and FolD, which catalyzes the two-step interconversion between 5,10-MeTHF and N^{10}-formyl-THF. The expression of these two enzymes during infection may boost regeneration of N^{10}-formyl-THF and 5,10-MeTHF needed for purine and dTMP synthesis (5, 143, 149). In addition, homologs encoding DHF reductase (Frd), which catalyzes the reduction of DHF to THF, have been found in cyanophages; coliphages; *Yersinia*-, *Salmonella*-, and *Pseudomonas*-infecting phages; and rumen viral metagenomes. Expression of phage Frd during infection may therefore help support production of dTMP by contributing to the regeneration of 5,10-MeTHF from DHF (1, 5, 35, 88, 109, 110, 112, 156, 170, 183). Finally, some viruses encode enzymes in the pathways synthesizing DHF and THF, especially GTP cyclohydrolase I (FolE), which catalyzes the first committed step in folate biosynthesis (5, 6, 62, 65, 109, 130, 144). Expression of phage-encoded one-carbon metabolism and folate synthesis enzymes together may constitute a strategy to enhance availability of one-carbon units and thus nucleotide biosynthesis in virocells.

4.2. Recycling of Host Nucleotides Complements De Novo Synthesis

Some virus-encoded enzymes can participate in both de novo nucleotide synthesis and host genome and RNA recycling. Viral ribonucleotide reductases, which convert NTPs to dNTPs, and the aforementioned thymidylate synthases are among the most widespread and best-known of these potentially dual-purpose enzymes, appearing in coliphages, vibriophages, cyanophages,

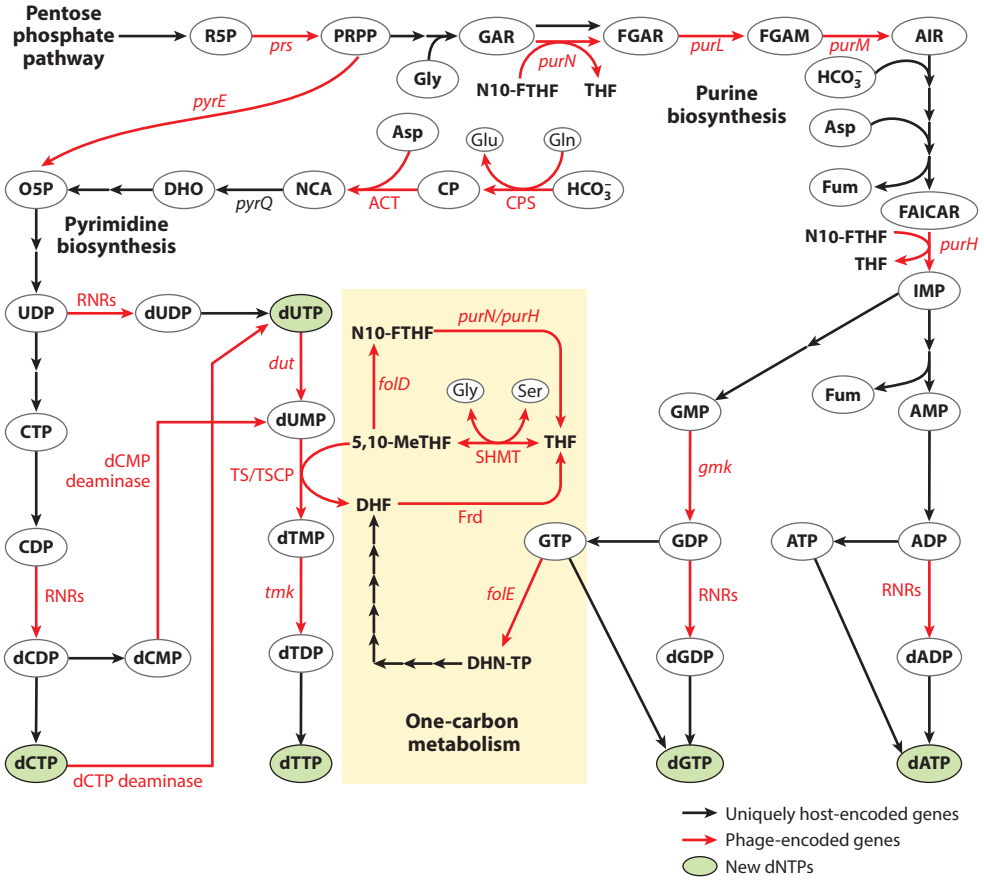


Figure 3

Phages usurp host nucleotide biosynthesis to generate viral biosynthetic capacity. Phages encode numerous de novo nucleotide biosynthesis genes (*red arrows*) to enhance production of new dNTPs (*green*). One-carbon metabolism, illustrated in the yellow box, is needed to generate necessary cofactors for purine and pyrimidine biosynthesis. Abbreviations: 5,10-MeTHF, 5,10-methylene-THF; ACT, aspartate carbamoyltransferase; AIR, 5-amino-1-(5-phospho-β-D-ribosyl)imidazole; CP, carbamoyl phosphate; CPS, CP synthetase; DHF, 7,8-dihydrofolate; DHN-TP, 7,8-dihydroneopterin 3′-triphosphate; DHO, dihydroorotate; *dut*, dUTP diphosphatase; FAICAR, 5-formamido-1-(5-phospho-D-ribosyl)-imidazole-4-carboxamide; FGAM, 2-(formamido)-N^1-(5-phospho-β-D-ribosyl)acetamidine; FGAR, N^2-formyl-GAR; *folD*, methylenetetrahydrofolate dehydrogenase; *folE*, GTP cyclohydrolase I; Frd, DHF reductase; Fum, fumarate; GAR, N^1-(5-phospho-β-D-ribosyl)glycinamide; *gmk*, guanylate kinase; IMP, inosine monophosphate; N10-FTHF, N^{10}-formyl-THF; NCA, *N*-carbamoyl-L-aspartate; O5P, orotidine 5-phosphate; PRPP, phosphoribosyl pyrophosphate; R5P, ribose 5-phosphate; RNR, ribonucleotide reductase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; *tmk*, thymidylate kinase; TS, thymidylate synthase; TSCP, TS-complementing protein.

and phage (meta)genomes from various environments (1, 10, 24, 26, 27, 38, 62, 66, 94, 108–110, 112, 113, 121, 157, 164, 170, 171, 179, 183). Some phages also encode dUTPases, which transform dUTP to dUMP, the substrate for thymidylate synthase and the thymidylate synthase isozyme TSCP (109, 110, 164, 170, 180, 185). As another example of a dual-purpose enzyme, PhiR1–37, which infects *Yersinia enterocolitica*, encodes a homolog of guanylate kinase (Gmk), which phosphorylates GMP to GDP, a necessary step in producing dGTP (89). GMP can be newly synthesized

or obtained from degradation of host RNAs. Many phages ensure access to dNTPs by encoding dNMP kinases (153, 170, 183), especially thymidylate kinases, which phosphorylate dTMP to dTDP and are present in cyanophages, *Pseudomonas* phages, and *Serratia* phages (1, 35, 54, 60, 164). Numerous phages of marine, freshwater, mammalian gut, and soil origin encode dCTP or dCMP deaminases, which respectively produce dUTP and dUMP, helping to convert the relatively overrepresented C and G bases from the host genome into A, T, and U bases, which are more prevalent in the phage genome (10, 26, 28, 35, 109, 112, 153, 157, 164, 179, 183, 185). Other phages, such as cyanophage S-PM2, degrade nucleotides that are overrepresented in the host genome relative to its own by encoding a MazG homolog that selectively degrades dGTP and dCTP (139).

During T4 phage infection of *Escherichia coli*, in perhaps the most thoroughly characterized example of phage participation in de novo nucleotide synthesis and recycling of the host genome, both ribonucleotide reductases and thymidylate synthases are incorporated into the T4 dNTP synthase complex. This complex incorporates both phage- and host-encoded proteins and provides not only hydroxymethyldeoxycytosine, a nucleotide unique to the phage genome, but also all other dNTPs in the same ratio in which they occur in the phage genome (106). As one mechanism to ensure appropriate levels of each nucleotide and incorporation of hydroxymethyldeoxycytosine in place of cytosine, T4 dUTPase also acts as a dCTPase and dCDPase, likely keeping dCTP levels low and preventing its incorporation into the T4 genome (83, 110). The T4 dNTP synthase complex produces dNTPs from both dNMPs released from host genome degradation and NDPs, created by host-encoded de novo nucleotide synthesis pathways, apparently through a metabolite-channeling mechanism that allows high pathway flux and delivery of dNTPs directly to phage replication forks. In addition, the complex integrates enzymes from nucleotide-salvage pathways, such as thymidine kinase, which phosphorylates thymidine to TMP (3, 109, 110).

4.3. Upregulation of Host Nucleotide Synthesis Genes

Instead of encoding nucleotide biosynthetic enzymes, some phages upregulate host purine and pyrimidine synthesis genes during infection. For example, infection of *P. aeruginosa* with LUZ19 results in upregulation of host *pyrQ*, which encodes dihydroorotase in the pyrimidine synthesis pathway (84), and SHMT, which participates in purine synthesis as described above. Similarly, infection of *Bacillus subtilis* with phi29 results in upregulation of host ribonucleotide reductases (111).

Infection of *P. aeruginosa* with phage phiKZ resulted in increased intracellular concentrations of many early metabolites in de novo nucleotide biosynthetic pathways, though phiKZ does not encode enzymes of the early steps in nucleotide synthesis (35). A similar result has been observed in phages of *Sulfitobacter* and other phages of *P. aeruginosa*, with virocells increasing or maintaining nucleotide concentrations during infection (7, 28, 35, 186). Additionally, phage PAK_P3 infection of *P. aeruginosa* leads to elevated concentrations of most dNTPs as infection progresses (28). Phage demand for nucleotides caused by rapid genome replication may help increase flux through synthesis pathways, but this cannot easily explain elevated levels of many intermediates in these pathways, which are likely caused by phage modulation of the abundance or activity of select enzymes (7, 28, 35, 63, 84, 186).

Viral hijacking of host nucleotide pathways is perhaps the single most widespread intrusion into host metabolic processes. Although the specific alterations to host nucleotide metabolism are diverse, they are all expected to help maintain an adequate supply of nucleotides during infection, and they likely involve direct modulation of nucleotide synthesis rates by virus-encoded enzymes or via modulation of host enzyme expression or activity. Importantly, viral control of nucleotide synthesis appears to be supported by alterations to other parts of metabolism, such as enhanced energy generation, modifications to central carbon metabolism that enhance synthesis

of nucleotide precursors, and improved assimilation of elemental nutrients (i.e., nitrogen and phosphorous) needed for nucleotide synthesis.

5. NUTRIENT ASSIMILATION: NITROGEN, PHOSPHATE, AND SULFUR

Access to nitrogen, phosphate, and sulfur is essential to successful viral replication. Though viruses can source these nutrients from the host cell, evidence indicates a reliance on extracellular sources (31, 155, 168, 173). Some viruses control host nutrient assimilation pathways, while others encode their own assimilatory mechanisms. In addition, some viruses make use of host nutrient-sensing regulatory proteins to control viral gene expression.

5.1. Nitrogen

Phage infection increases nitrogen demand due to the relatively high protein and nucleic acid content of viral particles relative to prokaryotic hosts (7, 168). Experiments with cyanophage S-SM1 and coliphage T6 have shown that phage particles incorporate nitrogen from both the host and the extracellular environment (82, 168), emphasizing the importance of continued nitrogen assimilation during infection.

To ensure access to nitrogen, some phages appear to be able to modulate nitrogen assimilation. For example, infection of *Sulfitobacter* with phage phi2047A results in a large increase in nitrogen uptake and cellular nitrogen-to-carbon ratio together with a rapid elevation of intracellular levels of many amino acids, including glutamate and glutamine, which are key nitrogen donors in biosynthetic pathways (7). In another example, an analysis of marine viral metagenomes revealed potential homologs of the ammonia transporter *amt*, often located near homologs of *glnB*, which encodes P-II (143). Bacterial P-II proteins are critical regulators of nitrogen metabolism, controlling the activity of glutamine synthetase, nitrate reduction, and high-affinity ammonia transport (8, 49, 50). Therefore, coexpression of viral *amt* and *glnB* during infection may increase ammonia assimilation by marine phages. Finally, some viruses appear to respond to host nutrient signaling pathways. For example, cyanophage genomes were found to contain binding sites for the nitrogen regulator NtcA, which upregulates transcription of its targets in response to accumulating α-ketoglutarate (157). Viral NtcA binding sites may thus allow the virus to respond to nitrogen availability in its hosts by tailoring its gene expression to nutrient availability.

Changes in intracellular amino acid levels appear common during infection, but this varies considerably across phages. For example, in contrast to infection of *Sulfitobacter* with phage phi2047A, infection of *P. aeruginosa* with phage YuA results in large decreases in amino acid concentrations. However, other *P. aeruginosa* phages decrease levels of only some amino acids, and infection of *P. aeruginosa* with phage phiKZ does not appear to depress amino acid levels at all (35). Interestingly, phages do not appear to encode enzymes exclusively involved in amino acid synthesis; the phage-encoded enzymes involved in amino acid synthesis identified so far also participate in other processes, such as one-carbon metabolism (see Section 4.1). Although amino acid availability is critical to virion production, it is possible that selective pressure to maintain a small genome precludes encoding the large number of enzymes required for amino acid synthesis. Instead, viruses may enhance amino acid synthesis by upregulating nitrogen assimilation as described above or by upregulating host amino acid synthesis genes. Indeed, transcriptomic and proteomic analyses during phage infection of *Pseudomonas*, *Bacteroidetes*, *Pseudoalteromonas*, and *Bacillus* species have all shown alterations in the abundance of host proteins and transcripts involved in amino acid synthesis, although integrated studies capable of quantitatively explaining metabolite changes in terms of enzyme expression are still needed (28, 63, 64, 84, 111, 186).

5.2. Phosphate

Though viruses can derive some of the necessary phosphate for viral genome replication from degradation of the host genome, access to extracellular phosphate has been shown to be important for rapid and effective reproduction in some phages (75, 176). Potentially as a means to increase access to phosphate during infection, marine viruses including cyanophages possess homologs of *pstS*, which encodes a phosphate-binding protein involved in phosphate transport (42, 75, 91, 108, 156, 157, 160, 175). Expression of this gene is high under phosphorous-depleted conditions, and under these low-phosphorous conditions it may decrease time to lysis (91, 176, 184). To regulate phosphate-acquisition genes, some cyanophage genomes contain *pho* box motifs upstream of some genes, allowing them to respond to phosphate abundance through the host PhoR/PhoB two-component regulatory system (75, 184). Some other phages, such as *Y. enterocolitica* phage phiR1–37, upregulate host *pstS*, along with other transport genes, instead of encoding its own transporter (89).

The phosphate metabolism genes *phoA* and *phoH* are also commonly found in viruses—including roseophages, coliphages, and cyanophages—though their roles are less clear (75, 104, 108, 109, 140, 156, 157, 170, 171, 175). PhoA is an alkaline phosphatase, presumably catalyzing the removal of phosphate groups from a variety of substrates to free bound phosphates for viral use (76, 145). PhoH is less well-characterized, though it may also act as a phosphatase (74, 78). Like *pstS*, *phoA* is upregulated under phosphorous-depleted conditions, though *phoH* is not, suggesting a potentially divergent role (160, 184). Nonetheless, *phoH* is prevalent in marine cyanophage genomes (157) and the genomes of many other prokaryotic viruses, suggesting an important role for this gene in viral infection (10, 89, 109, 121, 144, 164, 170, 180, 183).

5.3. Sulfur

Many prokaryotic viruses upregulate host sulfur assimilatory processes or have putative enzymes involved in sulfur assimilation. For example, phages infecting *Burkholderia* spp., *Vibrio* spp., and freshwater cyanobacteria, as well as human gut and cow rumen viral metagenomes, contain homologs of phosphoadenosine phosphosulfate reductases that participate in the assimilatory sulfate reduction pathway (5, 54, 97, 138, 159). It has also been observed that adenylyl-sulfate kinase, another component of the assimilatory sulfate reduction pathway, is present in a phage of *Ralstonia solanacearum* as well as in marine viral metagenomes (42, 179). Demonstrating another potential mechanism of sulfur assimilation, a phage isolated on a marine alphaproteobacterium was found to encode a putative subunit of methanesulfonate monooxygenase, which provides access to methanesulfonic acid, an abundant environmental sulfur source; however, the phage gene did not encode some of the substrate-binding residues (72). During infection, virus-encoded sulfur assimilatory enzymes may help maintain a steady supply of this nutrient, likely supporting biogenesis of iron-sulfur proteins that participate in electron transport chains and other redox processes. Along the same lines, genes for putative cysteine desulfurases, which participate in the biogenesis and loading of iron-sulfur clusters into proteins, have been found in viral metagenomes originating in marine environments, the human gut, and the bovine rumen, suggesting a viral role in iron-sulfur cluster biogenesis (5, 138, 149). Viral encoding of these proteins may therefore help maintain energy generation via electron transport chains.

Instead of encoding enzymes directly involved in sulfur assimilatory pathways, some cyanophages may support sulfur assimilation by encoding Fds that redirect electrons to these pathways. As discussed above (Section 2.1), cyanophage-encoded Fds are most similar to the cyanobacterial Fds used to provide electrons for nutrient assimilation. Heterologous coexpression of cyanophage Fd together with a *Prochlorococcus marinus* assimilatory sulfite reductase

and an FNR restored sulfur assimilation in *E. coli*, supporting a potential role in nutrient acquisition for phage Fds. This role may extend to other nutrients, such as nitrogen acquisition by nitrite reductase or providing electrons to phage synthetic enzymes, such as heme oxygenase (20, 34, 86, 87). Finally, instead of encoding its own enzymes, an HP1 infection of *Pseudoalteromonas* sp. resulted in upregulation of the host assimilatory sulfate reduction and cysteine synthesis pathway. The viral proteome is not enriched for cysteine relative to the host, suggesting the cysteine was not needed for viral protein production; instead, the assimilated sulfur was used by the virocell (64).

6. TOWARD A QUANTITATIVE UNDERSTANDING OF METABOLIC REMODELING DURING VIRAL INFECTION

Isotope-based metabolic flux analysis is a powerful tool for assessing in vivo pathway activity that can be used to determine metabolic network structure, intracellular fluxes, and pathway thermodynamics (47, 53, 68, 69, 85, 122, 124, 128, 129, 177). This technique has been applied in a wide range of prokaryotic systems to investigate changes in metabolic activity during developmental processes and in response to genetic and environmental perturbations (29, 36, 57, 115, 120, 127, 132, 178).

As we have discussed throughout this review, an ever-increasing wealth of genomic and metagenomic data provide a clear indication that prokaryotic viruses hijack a wide range of host metabolic activities to aid their reproduction (see the sidebar titled Metabolic Remodeling by Lysogens). However, experimental validation of the many predictions originating from this data is just beginning. We anticipate that metabolic flux analysis, in combination with other systems-level approaches such as quantitative metabolomics and shotgun proteomics, can provide a comprehensive and quantitative understanding of how host metabolic activity is dynamically reprogrammed during viral infection.

For example, ^{13}C metabolic flux analysis during viral infection of cyanobacteria could be used to directly measure cyanophage-induced shutdown of the Calvin cycle, mobilization of glycogen

METABOLIC REMODELING BY LYSOGENS

While this review is focused on virus-induced metabolic alterations that ostensibly occur during the lytic cycle, viral lysogeny can also alter host metabolism and overall physiology. Perhaps the best-known examples of lysogenic conversion involve phage-encoded virulence factors that enhance pathogenesis in lysogenized hosts; for example, vibriophage CTXphi encodes the cholera toxin, and *Salmonella enterica* phage Gifsy-2 encodes a superoxide dismutase that protects lysogens from the oxidative burst produced by the host immune response (9, 18, 21, 23, 46, 98, 102, 126, 131, 133, 169, 174).

Besides influencing virulence, temperate phages can alter many other physiological characteristics of their hosts, including growth rate, stress resistance, sporulation, colony morphology, exopolysaccharide synthesis, aggregation, and motility (9, 25, 40, 71, 90, 102, 126, 147). For example, under aerobic conditions, *Escherichia coli* phage HK022 suppresses the normally stochastic expression of the host machinery required for use of trimethylamine oxide (TMAO) as an alternate electron acceptor, resulting in a slower transition to TMAO use when oxygen is depleted (22).

The examples discussed here exemplify only a fraction of the strategies employed by temperate phages to manipulate host metabolism (18, 102, 126, 174). Phages capable of either temperate or lytic life cycles manipulate host processes to enhance their own survival and reproduction. However, the relatively long-term association of temperate phages with their hosts may favor a temporal alignment of host and virus goals.

Enzyme efficiency: net flux carried per unit of enzyme

reserves, increased flux through the oxidative and nonoxidative PPPs, and redirection of carbon toward nucleotide synthesis pathways. Isotope tracer experiments have already been used to determine flux distributions in uninfected cyanobacteria and mutants, demonstrating the feasibility of such an approach (59). In genetically tractable systems, or in cases where closely related phages differ by only one or a few relevant genes, isotope tracer studies may be used to investigate the effects of specific viral genes in altering host metabolism by comparing network structure and fluxes during infection across different phages. Time-dependent changes in metabolite levels and fluxes can be matched against changes in protein levels, of either host or viral origin, obtained from shotgun proteomics to help identify the key enzymes responsible for specific changes in pathway flux (85, 123, 132, 178).

As metabolite concentrations, fluxes, enzyme levels, and pathway thermodynamics are all interdependent, quantitative data from the aforementioned multi-omics experiments can be used to develop computational models that provide insight into the mechanisms underlying metabolic remodeling during viral infection (39, 47, 122, 124). Importantly, the kinetic parameters and allosteric regulators of phage-encoded enzymes may differ significantly from their host enzyme counterparts (163); thus, measurement of these parameters using in vitro biochemical assays may prove critical for the construction of accurate computational models with predictive power. In a few instances, computational approaches have been applied to model changes in intracellular fluxes during infection, but these attempts did not incorporate metabolomic, flux, or proteomic data (14, 15, 58, 70, 182).

Thermodynamics constitutes a key determinant of flux and enzyme efficiency in metabolic networks. A biochemical reaction with a strong thermodynamic driving force (i.e., with a large negative ΔG) will achieve a higher net flux given a fixed amount of enzyme than one closer to equilibrium (47, 122, 124, 125). Within a pathway, steps closer to equilibrium will be the least enzyme-efficient. Considering the selective pressure that phages face to maintain small genome and thus proteome sizes, the most cost-effective strategy to increase flux through a given metabolic pathway would be to encode or upregulate enzymes catalyzing the most thermodynamically favorable reactions within the pathway (47, 122, 124, 125). This makes virocell metabolism especially suited for modeling using the max-min driving force algorithm, whose objective function minimizes the thermodynamic limitations in a pathway, thereby maximizing the flux per enzyme (122, 124).

Use of modeling techniques to understand phage manipulation of metabolism is especially relevant considering that phages are increasingly recognized as major players in biogeochemically relevant processes. Whereas microbes have long been known to contribute to these processes, viruses have been identified more recently as playing major roles in manipulating nutrient fluxes given the ubiquity of viral infection in some environments; the effect of viruses on the composition of cell lysates; and the expanding list of viral genes involved in important processes such as nutrient uptake, regulation of carbon fixation, photosynthesis, and sulfur and ammonia oxidation (4, 5, 7, 16, 17, 41, 52, 77, 99, 137, 142, 143, 187). For example, cyanophages limit carbon fixation in infected cyanobacteria while maintaining photophosphorylation and nutrient uptake (52, 135, 137, 168). Considering that the viral infection rate in cyanobacteria may exceed 50%, phage-induced reduction in carbon fixation may have global effects (137, 150). Even where they do not directly impact nutrient cycling, viruses may support microbial communities that do; modeling of a small microbiome revealed that viral metabolic genes may play a role in providing communities with necessary metabolites by encoding enzymes or pathways that would otherwise be a bottleneck for the community (67). Despite the potential importance of viruses to relevant biogeochemical processes, current models often do not account for the effects of viral infection or manipulation of host processes. A thorough understanding of global nutrient cycling likely must incorporate the

contribution of viruses, requiring characterization of both viral effects on host metabolic processes and viral abundance.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This study was supported by National Science Foundation (NSF) grant award number 1715710.

LITERATURE CITED

1. Adriaenssens EM, Mattheus W, Cornelissen A, Shaburova O, Krylov VN, et al. 2012. Complete genome sequence of the giant *Pseudomonas* phage Lu11. *J. Virol.* 86(11):6369–70
2. Ahlgren NA, Fuchsman CA, Rocap G, Fuhrman JA. 2019. Discovery of several novel, widespread, and ecologically distinct marine *Thaumarchaeota* viruses that encode *amoC* nitrification genes. *ISME J.* 13:618–31
3. Allen JR, Lasser GW, Goldman DA, Booth JW, Mathews CK. 1983. T4 phage deoxyribonucleotide-synthesizing enzyme complex. *J. Biol. Chem.* 258(9):5746–53
4. Anantharaman K, Duhaime MB, Breier JA, Wendt KA, Toner BM, Dick GJ. 2014. Sulfur oxidation genes in diverse deep-sea viruses. *Science* 344(6185):757–60
5. Anderson CL, Sullivan MB, Fernando SC. 2017. Dietary energy drives the dynamic response of bovine rumen viral communities. *Microbiome* 5(1):155
6. Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, et al. 2006. The marine viromes of four oceanic regions. *PLOS Biol.* 4(11):2121–31
7. Ankrah NYD, May AL, Middleton JL, Jones DR, Hadden MK, et al. 2014. Phage infection of an environmentally relevant marine bacterium alters host metabolism and lysate composition. *ISME J.* 8(5):1089–100
8. Arcondéguy T, Jack R, Merrick M. 2001. P_{II} signal transduction proteins, pivotal players in microbial nitrogen control. *Microbiol. Mol. Biol. Rev.* 65(1):80–105
9. Askora A, Kawasaki T, Fujie M, Yamada T. 2017. Lysogenic conversion of the phytopathogen *Ralstonia solanacearum* by the P2virus ΦRSY1. *Front. Microbiol.* 8:2212
10. Bai M, Cheng YH, Sun XQ, Wang ZY, Wang YX, et al. 2019. Nine novel phages from a plateau lake in southwest China: insights into *Aeromonas* phage diversity. *Viruses* 11(7):615
11. Bailey S, Clokie MRJ, Millard A, Mann NH. 2004. Cyanophage infection and photoinhibition in marine cyanobacteria. *Res. Microbiol.* 155:720–25
12. Bailey S, Melis A, Mackey KRM, Cardol P, Finazzi G, et al. 2008. Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*. *Biochim. Biophys. Acta Bioenerg.* 1777:269–76
13. Berg BL, Baron C, Stewart V. 1991. Nitrate-inducible formate dehydrogenase in *Escherichia coli* K-12: I. Nucleotide sequence of the *fdnGHI* operon and evidence that opal (UGA) encodes selenocysteine. *J. Biol. Chem.* 266(33):22380–85
14. Birch EW, Ruggero NA, Covert MW. 2012. Determining host metabolic limitations on viral replication via integrated modeling and experimental perturbation. *PLOS Comput. Biol.* 8(10):e1002746
15. Bragg JG, Chisholm SW. 2008. Modeling the fitness consequences of a cyanophage-encoded photosynthesis gene. *PLOS ONE* 3(10):e3550
16. Breitbart M. 2012. Marine viruses: truth or dare. *Annu. Rev. Mar. Sci.* 4:425–48
17. Breitbart M, Bonnain C, Malki K, Sawaya NA. 2018. Phage puppet masters of the marine microbial realm. *Nat. Microbiol.* 3(7):754–66
18. Brüssow H, Canchaya C, Hardt W-D. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev.* 68(3):560–602

19. Busch AWU, Reijerse EJ, Lubitz W, Hofmann E, Frankenberg-Dinkel N. 2011. Radical mechanism of cyanophage phycoerythrobilin synthase (PebS). *Biochem. J.* 433(3):469–76
20. Campbell IJ, Olmos JL Jr., Xu W, Kahanda D, Atkinson JT, et al. 2020. *Prochlorococcus* phage ferredoxin: structural characterization and electron transfer to cyanobacterial sulfite reductases. *J. Biol. Chem.* 295:10610–23
21. Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brüssow H. 2003. Phage as agents of lateral gene transfer. *Curr. Opin. Microbiol.* 6(4):417–24
22. Carey JN, Mettert EL, Fishman-Engel DR, Roggiani M, Kiley PJ, Goulian M. 2019. Phage integration alters the respiratory strategy of its host. *eLife* 8:e49081
23. Casjens S. 2003. Prophages and bacterial genomics: What have we learned so far? *Mol. Microbiol.* 49(2):277–300
24. Chen F, Lu J. 2002. Genomic sequence and evolution of marine cyanophage P60: a new insight on lytic and lysogenic phages. *Appl. Environ. Microbiol.* 68(5):2589–94
25. Chen Y, Golding I, Sawai S, Guo L, Cox EC. 2005. Population fitness and the regulation of *Escherichia coli* genes by bacterial viruses. *PLOS Biol.* 3(7):1276–82
26. Chénard C, Chan AM, Vincent WF, Suttle CA. 2015. Polar freshwater cyanophage S-EIV1 represents a new widespread evolutionary lineage of phages. *ISME J.* 9:2046–58
27. Cheng M, Luo M, Xi H, Zhao Y, Le S, et al. 2020. The characteristics and genome analysis of vB_ApiP_XC38, a novel phage infecting *Acinetobacter pittii. Virus Genes* 56(4):498–507
28. Chevallereau A, Blasdel BG, De Smet J, Monot M, Zimmermann M, et al. 2016. Next-generation "-omics" approaches reveal a massive alteration of host RNA metabolism during bacteriophage infection of *Pseudomonas aeruginosa*. *PLOS Genet.* 12(7):e1006134
29. Christodoulou D, Link H, Fuhrer T, Kochanowski K, Gerosa L, Sauer U. 2018. Reserve flux capacity in the pentose phosphate pathway enables *Escherichia coli*'s rapid response to oxidative stress. *Cell Syst.* 6(5):569–78.e7
30. Clokie MRJ, Shan J, Bailey S, Jia Y, Krisch HM, et al. 2006. Transcription of a "photosynthetic" T4-type phage during infection of a marine cyanobacterium. *Environ. Microbiol.* 8(5):827–35
31. Cohen SS. 1948. The synthesis of bacterial viruses: II. The origin of the phosphorus found in the desoxyribonucleic acids of the T_2 and T_4 bacteriophages. *J. Biol. Chem.* 174:295–304
32. Collier JL, Grossman AR. 1992. Chlorosis induced by nutrient deprivation in *Synechococcus* sp. strain PCC 7942: Not all bleaching is the same. *J. Bacteriol.* 174(14):4718–26
33. Collier JL, Grossman AR. 1994. A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. *EMBO J.* 13(5):1039–47
34. Dammeyer T, Bagby SC, Sullivan MB, Chisholm SW, Frankenberg-Dinkel N. 2008. Efficient phage-mediated pigment biosynthesis in oceanic cyanobacteria. *Curr. Biol.* 18(6):442–48
35. De Smet J, Zimmermann M, Kogadeeva M, Ceyssens P-J, Vermaelen W, et al. 2016. High coverage metabolomics analysis reveals phage-specific alterations to *Pseudomonas aeruginosa* physiology during infection. *ISME J.* 10:1823–35
36. Dinh HV, Suthers PF, Chan SHJ, Shen Y, Xiao T, et al. 2019. A comprehensive genome-scale model for *Rhodosporidium toruloides* IFO0880 accounting for functional genomics and phenotypic data. *Metab. Eng. Commun.* 9:e00101
37. Doello S, Klotz A, Makowka A, Gutekunst K, Forchhammer K. 2018. A specific glycogen mobilization strategy enables rapid awakening of dormant cyanobacteria from chlorosis. *Plant Physiol.* 177(2):594–603
38. Doron S, Fedida A, Hernández-Prieto MA, Sabehi G, Karunker I, et al. 2016. Transcriptome dynamics of a broad host-range cyanophage and its hosts. *ISME J.* 10(6):1437–55
39. Du B, Zielinski DC, Monk JM, Palsson BO. 2018. Thermodynamic favorability and pathway yield as evolutionary tradeoffs in biosynthetic pathway choice. *PNAS* 115(44):11339–44
40. Edlin G, Lin L, Bitner R. 1977. Reproductive fitness of P1, P2, and Mu lysogens of *Escherichia coli*. *J. Virol.* 21(2):560–64
41. Emerson JB, Roux S, Brum JR, Bolduc B, Woodcroft BJ, et al. 2018. Host-linked soil viral ecology along a permafrost thaw gradient. *Nat. Microbiol.* 3(8):870–80
42. Enav H, Mandel-Gutfreund Y, Béjà O. 2014. Comparative metagenomic analyses reveal viral-induced shifts of host metabolism towards nucleotide biosynthesis. *Microbiome* 2(1):9

43. Ermakova M, Huokko T, Richaud P, Bersanini L, Howe CJ, et al. 2016. Distinguishing the roles of thylakoid respiratory terminal oxidases in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Physiol.* 171(2):1307–19
44. Farrant GK, Doré H, Cornejo-Castillo FM, Partensky F, Ratin M, et al. 2016. Delineating ecologically significant taxonomic units from global patterns of marine picocyanobacteria. *PNAS* 113(24):E3365–74
45. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* 281:237–40
46. Figueroa-Bossi N, Bossi L. 1999. Inducible prophages contribute to *Salmonella* virulence in mice. *Mol. Microbiol.* 33(1):167–76
47. Flamholz A, Noor E, Bar-Even A, Liebermeister W, Milo R. 2013. Glycolytic strategy as a tradeoff between energy yield and protein cost. *PNAS* 110(24):10039–44
48. Flombaum P, Gallegos JL, Gordillo RA, Rincón J, Zabala LL, et al. 2013. Present and future global distributions of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *PNAS* 110(24):9824–29
49. Forchhammer K. 2004. Global carbon/nitrogen control by P_{II} signal transduction in cyanobacteria: from signals to targets. *FEMS Microbiol. Rev.* 28(3):319–33
50. Forchhammer K, de Marsac NT. 1995. Functional analysis of the phosphoprotein P_{II} (*glnB* gene product) in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J. Bacteriol.* 177(8):2033–40
51. Forterre P. 2013. The virocell concept and environmental microbiology. *ISME J.* 7:233–36
52. Fridman S, Flores-Uribe J, Larom S, Alalouf O, Liran O, et al. 2017. A myovirus encoding both photosystem I and II proteins enhances cyclic electron flow in infected *Prochlorococcus* cells. *Nat. Microbiol.* 2(10):1350–57
53. Fuhrer T, Fischer E, Sauer U. 2005. Experimental identification and quantification of glucose metabolism in seven bacterial species. *J. Bacteriol.* 187(5):1581–90
54. Gao E-B, Gui J-F, Zhang Q-Y. 2012. A novel cyanophage with a cyanobacterial nonbleaching protein A gene in the genome. *J. Virol.* 86(1):236–45
55. Gasper R, Schwach J, Hartmann J, Holtkamp A, Wiethaus J, et al. 2017. Distinct features of cyanophage-encoded T-type phycobiliprotein lyase ΦCpeT: the role of auxiliary metabolic genes. *J. Biol. Chem.* 292(8):3089–98
56. Grossman AR, Schaefer MR, Chiang GG, Collier JL. 1993. The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiol. Rev.* 57(3):725–49
57. Haverkorn van Rijsewijk BRB, Nanchen A, Nallet S, Kleijn RJ, Sauer U. 2011. Large-scale ^{13}C-flux analysis reveals distinct transcriptional control of respiratory and fermentative metabolism in *Escherichia coli*. *Mol. Syst. Biol.* 7:477
58. Hellweger FL. 2009. Carrying photosynthesis genes increases ecological fitness of cyanophage *in silico*. *Environ. Microbiol.* 11(6):1386–94
59. Hendry JI, Prasannan C, Ma F, Möllers KB, Jaiswal D, et al. 2017. Rerouting of carbon flux in a glycogen mutant of cyanobacteria assessed via isotopically non-stationary ^{13}C metabolic flux analysis. *Biotechnol. Bioeng.* 114(10):2298–308
60. Hertveldt K, Lavigne R, Pleteneva E, Sernova N, Kurochkina L, et al. 2005. Genome comparison of *Pseudomonas aeruginosa* large phages. *J. Mol. Biol.* 354(3):536–45
61. Hess WR, Rocap G, Ting CS, Larimer F, Stilwagen S, et al. 2001. The photosynthetic apparatus of *Prochlorococcus*: insights through comparative genomics. *Photosynth. Res.* 70(1):53–71
62. Holmfeldt K, Solonenko N, Shah M, Corrier K, Riemann L, et al. 2013. Twelve previously unknown phage genera are ubiquitous in global oceans. *PNAS* 110(31):12798–803
63. Howard-Varona C, Hargreaves KR, Solonenko NE, Markillie LM, White RA, et al. 2018. Multiple mechanisms drive phage infection efficiency in nearly identical hosts. *ISME J.* 12(6):1605–18
64. Howard-Varona C, Lindback MM, Bastien GE, Solonenko N, Zayed AA, et al. 2020. Phage-specific metabolic reprogramming of virocells. *ISME J.* 14(4):881–95
65. Howard-Varona C, Roux S, Dore H, Solonenko NE, Holmfeldt K, et al. 2017. Regulation of infection efficiency in a globally abundant marine *Bacteriodetes* virus. *ISME J.* 11(1):284–95
66. Hurwitz BL, Hallam SJ, Sullivan MB. 2013. Metabolic reprogramming by viruses in the sunlit and dark ocean. *Genome Biol.* 14(11):R123

67. Islam MM, Fernando SC, Saha R. 2019. Metabolic modeling elucidates the transactions in the rumen microbiome and the shifts upon virome interactions. *Front. Microbiol.* 10:2412
68. Jacobson TB, Adamczyk PA, Stevenson DM, Regner M, Ralph J, et al. 2019. ^{2}H and ^{13}C metabolic flux analysis elucidates *in vivo* thermodynamics of the ED pathway in *Zymomonas mobilis*. *Metab. Eng.* 54:301–16
69. Jacobson TB, Korosh TK, Stevenson DM, Foster C, Maranas C, et al. 2020. *In vivo* thermodynamic analysis of glycolysis in *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* using ^{13}C and ^{2}H tracers. *mSystems* 5(2):e00736-19
70. Jain R, Srivastava R. 2009. Metabolic investigation of host/pathogen interaction using MS2-infected *Escherichia coli*. *BMC Syst. Biol.* 3:121
71. Jian H, Xiao X, Wang F. 2013. Role of filamentous phage SW1 in regulating the lateral flagella of *Shewanella piezotolerans* strain WP3 at low temperatures. *Appl. Environ. Microbiol.* 79(22):7101–9
72. Kang I, Oh HM, Kang D, Cho JC. 2013. Genome of a SAR116 bacteriophage shows the prevalence of this phage type in the oceans. *PNAS* 110(30):12343–48
73. Kaplan A. 2016. Cyanophages: starving the host to recruit resources. *Curr. Biol.* 26(12):R511–13
74. Kazakov AE, Vassieva O, Gelfand MS, Osterman A, Overbeek R. 2003. Bioinformatics classification and functional analysis of PhoH homologs. *In Silico Biol.* 3(1–2):3–15
75. Kelly L, Ding H, Huang KH, Osburne MS, Chisholm SW. 2013. Genetic diversity in cultured and wild marine cyanomyoviruses reveals phosphorus stress as a strong selective agent. *ISME J.* 7:1827–41
76. Kim EE, Wyckoff HW. 1991. Reaction mechanism of alkaline phosphatase based on crystal structures: two-metal ion catalysis. *J. Mol. Biol.* 218:449–64
77. Kim J-G, Kim S-J, Cvirkaite-Krupovic V, Yu W-J, Gwak J-H, et al. 2019. Spindle-shaped viruses infect marine ammonia-oxidizing thaumarchaea. *PNAS* 116(31):15645–50
78. Kim SK, Makino K, Amemura M, Shinagawa H, Nakata A. 1993. Molecular analysis of the *phoH* gene, belonging to the phosphate regulon in *Escherichia coli*. *J. Bacteriol.* 175(5):1316–24
79. Klughammer B, Sültemeyer D, Badger MR, Price GD. 1999. The involvement of NAD(P)H dehydrogenase subunits, NdhD3 and NdhF3, in high-affinity CO_2 uptake in *Synechococcus* sp. PCC7002 gives evidence for multiple NDH-1 complexes with specific roles in cyanobacteria. *Mol. Microbiol.* 32(6):1305–15
80. Komenda J, Sobotka R. 2016. Cyanobacterial high-light-inducible proteins—protectors of chlorophyll-protein synthesis and assembly. *Biochim. Biophys. Acta Bioenerg.* 1857(3):288–95
81. Kowalchuk GA, Stephen JR. 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Annu. Rev. Microbiol.* 55:485–529
82. Kozloff LM, Knowlton K, Putnam FW, Evans EA Jr. 1950. Biochemical studies of virus reproduction: V. The origin of bacteriophage nitrogen. *J. Biol. Chem.* 188(1):101–16
83. Kutter E, Bryan D, Ray G, Brewster E, Blasdel B, Guttman B. 2018. From host to phage metabolism: hot tales of phage T4's takeover of *E. coli*. *Viruses* 10(7):387
84. Lavigne R, Lecoutere E, Wagemans J, Cenens W, Aertsen A, et al. 2013. A multifaceted study of *Pseudomonas aeruginosa* shutdown by virulent podovirus LUZ19. *mBio* 4(2):e00061-13
85. Lawson CE, Nuijten GHL, de Graaf RM, Jacobson TB, Pabst M, et al. 2021. Autotrophic and mixotrophic metabolism of an anammox bacterium revealed by in vivo ^{13}C and ^{2}H metabolic network mapping. *ISME J.* 15:673–87
86. Ledermann B, Béjà O, Frankenberg-Dinkel N. 2016. New biosynthetic pathway for pink pigments from uncultured oceanic viruses. *Environ. Microbiol.* 18(12):4337–47
87. Ledermann B, Schwan M, Sommerkamp JA, Hofmann E, Béjà O, Frankenberg-Dinkel N. 2018. Evolution and molecular mechanism of four-electron reducing ferredoxin-dependent bilin reductases from oceanic phages. *FEBS J.* 285:339–56
88. Lee JY, Li Z, Miller ES. 2017. *Vibrio* phage KVP40 encodes a functional NAD^{+} salvage pathway. *J. Bacteriol.* 199(9):e00855-16
89. Leskinen K, Blasdel BG, Lavigne R, Skurnik M. 2016. RNA-sequencing reveals the progression of phage-host interactions between φR1–37 and *Yersinia enterocolitica*. *Viruses* 8(4):111
90. Lin L, Bitner R, Edlin G. 1977. Increased reproductive fitness of *Escherichia coli* lambda lysogens. *J. Virol.* 21(2):554–59

91. Lin X, Ding H, Zeng Q. 2016. Transcriptomic response during phage infection of a marine cyanobacterium under phosphorus-limited conditions. *Environ. Microbiol.* 18(2):450–60
92. Lindell D, Jaffe JD, Coleman ML, Futschik ME, Axmann IM, et al. 2007. Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. *Nature* 449:83–86
93. Lindell D, Jaffe JD, Johnson ZI, Church GM, Chisholm SW. 2005. Photosynthesis genes in marine viruses yield proteins during host infection. *Nature* 438(7064):86–89
94. Lindell D, Sullivan MB, Johnson ZI, Tolonen AC, Rohwer F, Chisholm SW. 2004. Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. *PNAS* 101(30):11013–18
95. Liu L-N. 2016. Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes. *Biochim. Biophys. Acta Bioenerg.* 1857:256–65
96. Liu R, Liu Y, Chen Y, Zhan Y, Zeng Q. 2019. Cyanobacterial viruses exhibit diurnal rhythms during infection. *PNAS* 116(28):14077–82
97. Liu Y, Zhao L, Wang M, Wang Q, Zhang X, et al. 2019. Complete genomic sequence of bacteriophage P23: a novel Vibrio phage isolated from the Yellow Sea, China. *Virus Genes* 55(6):834–42
98. Lopez CA, Winter SE, Rivera-Chávez F, Xavier MN, Poon V, et al. 2012. Phage-mediated acquisition of a Type III secreted effector protein boosts growth of *Salmonella* by nitrate respiration. *mBio* 3(3):e00143-12
99. Ma X, Coleman ML, Waldbauer JR. 2018. Distinct molecular signatures in dissolved organic matter produced by viral lysis of marine cyanobacteria. *Environ. Microbiol.* 20(8):3001–11
100. Maderbocus R, Fields BL, Hamilton K, Luo S, Tran TH, et al. 2017. Crystal structure of a *Pseudomonas* malonate decarboxylase holoenzyme hetero-tetramer. *Nat. Commun.* 8(1):160
101. Mahmoudabadi G, Milo R, Phillips R. 2017. Energetic cost of building a virus. *PNAS* 114(22):E4324–33
102. Mai-Prochnow A, Hui JGK, Kjelleberg S, Rakonjac J, McDougald D, Rice SA. 2015. 'Big things in small packages: the genetics of filamentous phage and effects on fitness of their host'. *FEMS Microbiol. Rev.* 39(4):465–87
103. Mallory JB, Alfano C, McMacken R. 1990. Host virus interactions in the initiation of bacteriophage λ DNA replication: recruitment of *Escherichia coli* DnaB helicase by λ P replication protein. *J. Biol. Chem.* 265(22):13297–307
104. Mann NH, Clokie MRJ, Millard A, Cook A, Wilson WH, et al. 2005. The genome of S-PM2, a "photosynthetic" T4-type bacteriophage that infects marine *Synechococcus* strains. *J. Bacteriol.* 187(9):3188–200
105. Mann NH, Cook A, Millard A, Bailey S, Clokie M. 2003. Bacterial photosynthesis genes in a virus. *Nature* 424(6950):741
106. Mathews CK. 1993. Enzyme organization in DNA precursor biosynthesis. *Prog. Nucleic Acid Res. Mol. Biol.* 44:167–203
107. Millard A, Clokie MRJ, Shub DA, Mann NH. 2004. Genetic organization of the *psbAD* region in phages infecting marine *Synechococcus* strains. *PNAS* 101(30):11007–12
108. Millard AD, Zwirglmaier K, Downey MJ, Mann NH, Scanlan DJ. 2009. Comparative genomics of marine cyanomyoviruses reveals the widespread occurrence of *Synechococcus* host genes localized to a hyperplastic region: implications for mechanisms of cyanophage evolution. *Environ. Microbiol.* 11(9):2370–87
109. Miller ES, Heidelberg JF, Eisen JA, Nelson WC, Durkin AS, et al. 2003. Complete genome sequence of the broad-host-range vibriophage KVP40: comparative genomics of a T4-related bacteriophage. *J. Bacteriol.* 185(17):5220–33
110. Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, Rüger W. 2003. Bacteriophage T4 genome. *Microbiol. Mol. Biol. Rev.* 67(1):86–156
111. Mojardín L, Salas M. 2016. Global transcriptional analysis of virus-host interactions between phage ϕ29 and *Bacillus subtilis*. *J. Virol.* 90(20):9293–304
112. Moreno Switt AI, Orsi RH, den Bakker HC, Vongkamjan K, Altier C, Wiedmann M. 2013. Genomic characterization provides new insight into *Salmonella* phage diversity. *BMC Genom.* 14:481
113. Morimoto D, Kimura S, Sako Y, Yoshida T. 2018. Transcriptome analysis of a bloom-forming cyanobacterium *Microcystis aeruginosa* during Ma-LMM01 phage infection. *Front. Microbiol.* 9:2
114. Müller AL, Kjeldsen KU, Rattei T, Pester M, Loy A. 2015. Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. *ISME J.* 9(5):1152–65

115. Murima P, Zimmermann M, Chopra T, Pojer F, Fonti G, et al. 2016. A rheostat mechanism governs the bifurcation of carbon flux in mycobacteria. *Nat. Commun.* 7:12527
116. Nadel O, Rozenberg A, Flores-Uribe J, Larom S, Schwarz R, Béjà O. 2019. An uncultured marine cyanophage encodes an active phycobilisome proteolysis adaptor protein NblA. *Environ. Microbiol. Rep.* 11(6):848–54
117. Nechaev S, Severinov K. 1999. Inhibition of *Escherichia coli* RNA polymerase by bacteriophage T7 gene 2 protein. *J. Mol. Biol.* 289:815–26
118. Nguyen AY, Bricker WP, Zhang H, Weisz DA, Gross ML, Pakrasi HB. 2017. The proteolysis adaptor, NblA, binds to the N-terminus of β-phycocyanin: implications for the mechanism of phycobilisome degradation. *Photosynth. Res.* 132(1):95–106
119. Ni T, Zeng Q. 2016. Diel infection of cyanobacteria by cyanophages. *Front. Mar. Sci.* 2:123
120. Nikel P, Fuhrer T, Chavarría M, Sánchez-Pascuala A, Sauer U, de Lorenzo V. 2020. Redox stress reshapes carbon fluxes of *Pseudomonas putida* for cytosolic glucose oxidation and NADPH generation. bioRxiv 2020.06.13.149542. **https://doi.org/10.1101/2020.06.13.149542**
121. Nilsson E, Li K, Fridlund J, Sulcius S, Bunse C, et al. 2019. Genomic and seasonal variations among aquatic phages infecting the Baltic Sea gammaproteobacterium *Rheinheimera* sp. strain BAL341. *Appl. Environ. Microbiol.* 85(18):e01003-19
122. Noor E, Bar-Even A, Flamholz A, Reznik E, Liebermeister W, Milo R. 2014. Pathway thermodynamics highlights kinetic obstacles in central metabolism. *PLOS Comput. Biol.* 10(2):e1003483
123. Noor E, Cherkaoui S, Sauer U. 2019. Biological insights through omics data integration. *Curr. Opin. Syst. Biol.* 15:39–47
124. Noor E, Flamholz A, Bar-Even A, Davidi D, Milo R, Liebermeister W. 2016. The protein cost of metabolic fluxes: prediction from enzymatic rate laws and cost minimization. *PLOS Comput. Biol.* 12(11):e1005167
125. Noor E, Flamholz A, Liebermeister W, Bar-Even A, Milo R. 2013. A note on the kinetics of enzyme action: a decomposition that highlights thermodynamic effects. *FEBS Lett.* 587:2772–77
126. Obeng N, Pratama AA, van Elsas JD. 2016. The significance of mutualistic phages for bacterial ecology and evolution. *Trends Microbiol.* 24(6):440–49
127. Olson DG, Hörl M, Fuhrer T, Cui J, Zhou J, et al. 2017. Glycolysis without pyruvate kinase in *Clostridium thermocellum*. *Metab. Eng.* 39:169–80
128. Park JO, Rubin SA, Xu YF, Amador-Noguez D, Fan J, et al. 2016. Metabolite concentrations, fluxes, and free energies imply efficient enzyme usage. *Nat. Chem. Biol.* 12(7):482–89
129. Park JO, Tanner LB, Wei MH, Khana DB, Jacobson TB, et al. 2019. Near-equilibrium glycolysis supports metabolic homeostasis and energy yield. *Nat. Chem. Biol.* 15:1001–8
130. Pedulla ML, Ford ME, Houtz JM, Karthikeyan T, Wadsworth C, et al. 2003. Origins of highly mosaic mycobacteriophage genomes. *Cell* 113(2):171–82
131. Peters DL, McCutcheon JG, Stothard P, Dennis JJ. 2019. Novel *Stenotrophomonas maltophilia* temperate phage DLP4 is capable of lysogenic conversion. *BMC Genom.* 20(1):300
132. Pisithkul T, Schroeder JW, Trujillo EA, Yeesin P, Stevenson DM, et al. 2019. Metabolic remodeling during biofilm development of *Bacillus subtilis*. *mBio* 10(3):e00623-19
133. Plunkett G III, Rose DJ, Durfee TJ, Blattner FR. 1999. Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: Shiga toxin as a phage late-gene product? *J. Bacteriol.* 181(6):1767–78
134. Pommier J, Mandrand MA, Holt SE, Boxer DH, Giordano G. 1992. A second phenazine methosulphate-linked formate dehydrogenase isoenzyme in *Escherichia coli*. *Biochem. Biophys. Acta Biomembr.* 1107(2):305–13
135. Puxty RJ, Evans DJ, Millard AD, Scanlan DJ. 2018. Energy limitation of cyanophage development: implications for marine carbon cycling. *ISME J.* 12:1273–86
136. Puxty RJ, Millard AD, Evans DJ, Scanlan DJ. 2015. Shedding new light on viral photosynthesis. *Photosynth. Res.* 126(1):71–97
137. Puxty RJ, Millard AD, Evans DJ, Scanlan DJ. 2016. Viruses inhibit CO_2 fixation in the most abundant phototrophs on Earth. *Curr. Biol.* 26(12):1585–89
138. Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, et al. 2010. Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* 466(7304):334–38

139. Rihtman B, Bowman-Grahl S, Millard A, Corrigan RM, Clokie MRJ, Scanlan DJ. 2019. Cyanophage MazG is a pyrophosphohydrolase but unable to hydrolyse magic spot nucleotides. *Environ. Microbiol. Rep.* 11(3):448–55
140. Rohwer F, Segall A, Steward G, Seguritan V, Breitbart M, et al. 2000. The complete genomic sequence of the marine phage Roseophage SIO1 shares homology with nonmarine phages. *Limnol. Oceanogr.* 45(2):408–18
141. Rokka A, Suorsa M, Saleem A, Battchikova N, Aro EM. 2005. Synthesis and assembly of thylakoid protein complexes: multiple assembly steps of photosystem II. *Biochem. J.* 388:159–68
142. Rosenwasser S, Ziv C, van Creveld SG, Vardi A. 2016. Virocell metabolism: metabolic innovations during host-virus interactions in the ocean. *Trends Microbiol.* 24(10):821–32
143. Roux S, Brum JR, Dutilh BE, Sunagawa S, Duhaime MB, et al. 2016. Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. *Nature* 537:689–93
144. Roux S, Enault F, Ravet V, Pereira O, Sullivan MB. 2015. Genomic characteristics and environmental distributions of the uncultivated Far-T4 phages. *Front. Microbiol.* 6:199
145. Santos-Beneit F. 2015. The Pho regulon: a huge regulatory network in bacteria. *Front. Microbiol.* 6:404
146. Sawa N, Tatsuke T, Ogawa A, Hirokawa Y, Osanai T, Hanai T. 2019. Modification of carbon metabolism in *Synechococcus elongatus* PCC 7942 by cyanophage-derived sigma factors for bioproduction improvement. *J. Biosci. Bioeng.* 127(2):256–64
147. Schuch R, Fischetti VA. 2009. The secret life of the anthrax agent *Bacillus anthracis*: bacteriophage-mediated ecological adaptations. *PLOS ONE* 4(8):e6532
148. Sharon I, Alperovitch A, Rohwer F, Haynes M, Glaser F, et al. 2009. Photosystem I gene cassettes are present in marine virus genomes. *Nature* 461(7261):258–62
149. Sharon I, Battchikova N, Aro E-M, Giglione C, Meinnel T, et al. 2011. Comparative metagenomics of microbial traits within oceanic viral communities. *ISME J.* 5(7):1178–90
150. Sharon I, Tzahor S, Williamson S, Shmoish M, Man-Aharonovich D, et al. 2007. Viral photosynthetic reaction center genes and transcripts in the marine environment. *ISME J.* 1(6):492–501
151. Shimakawa G, Hasunuma T, Kondo A, Matsuda M, Makino A, Miyake C. 2014. Respiration accumulates Calvin cycle intermediates for the rapid start of photosynthesis in *Synechocystis* sp. PCC 6803. *Biosci. Biotechnol. Biochem.* 78(12):1997–2007
152. Shinde S, Zhang X, Singapuri SP, Kalra I, Liu X, et al. 2020. Glycogen metabolism supports photosynthesis start through the oxidative pentose phosphate pathway in cyanobacteria. *Plant Physiol.* 182(1):507–17
153. Smith MCM, Burns RN, Wilson SE, Gregory MA. 1999. The complete genome sequence of the *Streptomyces* temperate phage ΦC31: evolutionary relationships to other viruses. *Nucleic Acids Res.* 27(10):2145–55
154. Stahl DA, de la Torre JR. 2012. Physiology and diversity of ammonia-oxidizing archaea. *Annu. Rev. Microbiol.* 66:83–101
155. Stent GS, Maaløe O. 1953. Radioactive phosphorus tracer studies on the reproduction of T_4 bacteriophage. *Biochim. Biophys. Acta* 10:55–69
156. Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW. 2005. Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. *PLOS Biol.* 3(5):e144
157. Sullivan MB, Huang KH, Ignacio-Espinoza JC, Berlin AM, Kelly L, et al. 2010. Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. *Environ. Microbiol.* 12(11):3035–56
158. Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, Chisholm SW. 2006. Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. *PLOS Biol.* 4(8):1344–57
159. Summer EJ, Gill JJ, Upton C, Gonzalez CF, Young R. 2007. Role of phages in the pathogenesis of *Burkholderia* or "Where are the toxin genes in *Burkholderia* phages?" *Curr. Opin. Microbiol.* 10(4):410–17
160. Tetu SG, Brahamsha B, Johnson DA, Tai V, Phillippy K, et al. 2009. Microarray analysis of phosphate regulation in the marine cyanobacterium *Synechococcus* sp. WH8102. *ISME J.* 3(7):835–49
161. Theis J, Schroda M. 2016. Revisiting the photosystem II repair cycle. *Plant Signal. Behav.* 11(9):e1218587

162. Thompson LR, Zeng Q, Chisholm SW. 2016. Gene expression patterns during light and dark infection of *Prochlorococcus* by cyanophage. *PLOS ONE* 11(10):e0165375
163. Thompson LR, Zeng Q, Kelly L, Huang KH, Singer AU, et al. 2011. Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. *PNAS* 108(39):E757–64
164. Tian C, Zhao J, Zhang Z, Chen X, Wei X, et al. 2019. Identification and molecular characterization of *Serratia marcescens* phages vB_SmaA_2050H1 and vB_SmaM_2050HW. *Arch. Virol.* 164(4):1085–94
165. Ting CS, Rocap G, King J, Chisholm SW. 2001. Phycobiliprotein genes of the marine photosynthetic prokaryote *Prochlorococcus*: evidence for rapid evolution of genetic heterogeneity. *Microbiology* 147(11):3171–82
166. Ting CS, Rocap G, King J, Chisholm SW. 2002. Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. *Trends Microbiol.* 10(3):134–42
167. Tolar BB, Herrmann J, Bargar JR, van den Bedem H, Wakatsuki S, Francis CA. 2017. Integrated structural biology and molecular ecology of N-cycling enzymes from ammonia-oxidizing archaea. *Environ. Microbiol. Rep.* 9(5):484–91
168. Waldbauer JR, Coleman ML, Rizzo AI, Campbell KL, Lotus J, Zhang L. 2019. Nitrogen sourcing during viral infection of marine cyanobacteria. *PNAS* 116(31):15590–95
169. Waldor MK, Mekalanos JJ. 1996. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 272(5270):1910–14
170. Wang J, Jiang Y, Vincent M, Sun Y, Yu H, et al. 2005. Complete genome sequence of bacteriophage T5. *Virology* 332(1):45–65
171. Weigele PR, Pope WH, Pedulla ML, Houtz JM, Smith AL, et al. 2007. Genomic and structural analysis of Syn9, a cyanophage infecting marine *Prochlorococcus* and *Synechococcus*. *Environ. Microbiol.* 9(7):1675–95
172. Welkie DG, Rubin BE, Diamond S, Hood RD, Savage DF, Golden SS. 2019. A hard day's night: cyanobacteria in diel cycles. *Trends Microbiol.* 27(3):231–42
173. Wikner J, Vallino JJ, Steward GF, Smith DC, Azam F. 1993. Nucleic acids from the host bacterium as a major source of nucleotides for three marine bacteriophages. *FEMS Microbiol. Ecol.* 12(4):237–48
174. Williamson KE, Fuhrmann JJ, Wommack KE, Radosevich M. 2017. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. *Annu. Rev. Virol.* 4:201–19
175. Williamson SJ, Rusch DB, Yooseph S, Halpern AL, Heidelberg KB, et al. 2008. The Sorcerer II Global Ocean Sampling Expedition: metagenomic characterization of viruses within aquatic microbial samples. *PLOS ONE* 3(1):e1456
176. Wilson WH, Carr NG, Mann NH. 1996. The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. *J. Phycol.* 32:506–16
177. Xiong W, Lo J, Chou KJ, Wu C, Magnusson L, et al. 2018. Isotope-assisted metabolite analysis sheds light on central carbon metabolism of a model cellulolytic bacterium *Clostridium thermocellum*. *Front. Microbiol.* 9:1947
178. Xu J, Martien J, Gilbertson C, Ma J, Amador-Noguez D, Park JO. 2020. Metabolic flux analysis and fluxomics-driven determination of reaction free energy using multiple isotopes. *Curr. Opin. Biotechnol.* 64:151–60
179. Yamada T, Satoh S, Ishikawa H, Fujiwara A, Kawasaki T, et al. 2010. A jumbo phage infecting the phytopathogen *Ralstonia solanacearum* defines a new lineage of the Myoviridae family. *Virology* 398(1):135–47
180. Yoshida T, Nagasaki K, Takashima Y, Shirai Y, Tomaru Y, et al. 2008. Ma-LMM01 infecting toxic *Microcystis aeruginosa* illuminates diverse cyanophage genome strategies. *J. Bacteriol.* 190(5):1762–72
181. Yoshida-Takashima Y, Yoshida M, Ogata H, Nagasaki K, Hiroishi S, Yoshida T. 2012. Cyanophage infection in the bloom-forming cyanobacteria *Microcystis aeruginosa* in surface freshwater. *Microbes Environ.* 27(4):350–55
182. You L, Suthers PF, Yin J. 2002. Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. *J. Bacteriol.* 184(7):1888–94
183. Yuan Y, Xi H, Dai J, Zhong Y, Lu S, et al. 2020. The characteristics and genome analysis of the novel *Y. pestis* phage JC221. *Virus Res.* 283:197982
184. Zeng Q, Chisholm SW. 2012. Marine viruses exploit their host's two-component regulatory system in response to resource limitation. *Curr. Biol.* 22(2):124–28

185. Zhang Y, Maley F, Maley GF, Duncan G, Dunigan DD, Van Etten JL. 2007. Chloroviruses encode a bifunctional dCMP-dCTP deaminase that produces two key intermediates in dTTP formation. *J. Virol.* 81(14):7662–71
186. Zhao X, Shen M, Jiang X, Shen W, Zhong Q, et al. 2017. Transcriptomic and metabolomics profiling of phage-host interactions between phage PaP1 and *Pseudomonas aeruginosa*. *Front. Microbiol.* 8:548
187. Zimmerman AE, Howard-Varona C, Needham DM, John SG, Worden AZ, et al. 2020. Metabolic and biogeochemical consequences of viral infection in aquatic ecosystems. *Nat. Rev. Microbiol.* 18:21–34
188. Zinser ER, Lindell D, Johnson ZI, Futschik ME, Steglich C, et al. 2009. Choreography of the transcriptome, photophysiology, and cell cycle of a minimal photoautotroph, *Prochlorococcus*. *PLOS ONE* 4(4):e5135