Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism

Luke R. Thompson[a,1], Qinglu Zeng[b,1], Libusha Kelly[b], Katherine H. Huang[b], Alexander U. Singer[c], JoAnne Stubbe[a,d,2], and Sallie W. Chisholm[a,b,2]

Departments of [a]Biology and [b]Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; [c]Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario Canada M5G 1L6; and [d]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

AUTHOR SUMMARY

Approximately half of all photosynthesis on Earth takes place in the ocean, and a sizable portion of that is carried out by tiny (about 1 μm in diameter) unicellular cyanobacteria, or blue-green algae, called *Prochlorococcus* and *Synechococcus*, which often reach densities of 100 million per liter of seawater. Even more abundant are viruses, called cyanophage, that can attach to these "host" cells, inject their DNA, and use the host's biochemical machinery to make more phage. The phage then break open the host cell, killing it, and release their progeny into the seawater. One of the ways cyanophages are thought to take over their hosts is via the use of host-like metabolic genes—acquired over eons of intimate coevolution—that encode enzymes that boost metabolic steps that might be bottlenecks in the phage reproduction process. An apparent contradiction arises, however, in that many cyanophages carry genes for both photosynthesis and a carbon metabolism pathway called the pentose phosphate pathway, and these two processes achieve opposite goals, with photosynthesis "fixing" carbon and the pentose phosphate pathway "burning" carbon. Are host-like metabolic genes in cyanophage steering host metabolism toward fixing carbon or toward burning carbon? In this work, we used a combination of physiology, enzymology, and sequencing approaches to address this question. Our evidence suggests that cyanophages direct host metabolism to mobilize carbon stores, burning but not fixing carbon to fuel the synthesis of DNA building blocks and phage replication.

The first clues to the strategy used by cyanophages for directing host metabolism came from the analysis of cyanophage genomes. These genomes revealed the widespread presence of the "auxiliary metabolic genes" mentioned earlier, which encode proteins similar to those used in host metabolism. These genes tend to be absent from viruses infecting noncyanobacterial hosts, suggesting that they are a specific adaptation suited to infecting cyanobacteria. The metabolic pathways that these phage genes are associated with in host cells include the light reactions of photosynthesis (1), the pentose phosphate pathway (1), nutrient acquisition pathways (2), and pathways for nucleotide biosynthesis that are also commonly found in noncyanophages (2).

To make sense of host cyanobacterial metabolism as it relates to phage infection, we first constructed a simple model of host metabolism (Fig. P1*A*), facilitated by an analysis of gene expression patterns over the light–dark cycle of *Prochlorococcus* (3). In this model, cyanobacteria use the light reactions of photosynthesis to harness light energy (*hv*) and split water to produce NADPH (electron carrier) and ATP (energy carrier), using these metabolites to fix CO_2 in the Calvin cycle (green) and produce glucose 6-phosphate during the day. At night, this sugar is oxidized in the pentose phosphate pathway (red) to NADPH and ribose 5-phosphate, which can be used for carbon skeletons or recycled back through the pathway. Finally, nucleotide biosynthesis takes the NADPH and ribose 5-phosphate produced by the pentose phosphate pathway, combined with NADPH and ATP produced by the light reactions of photosynthesis, to produce nucleotides, the DNA building blocks of chromosomes. In essence, taking energy from the sun, electrons from water, and

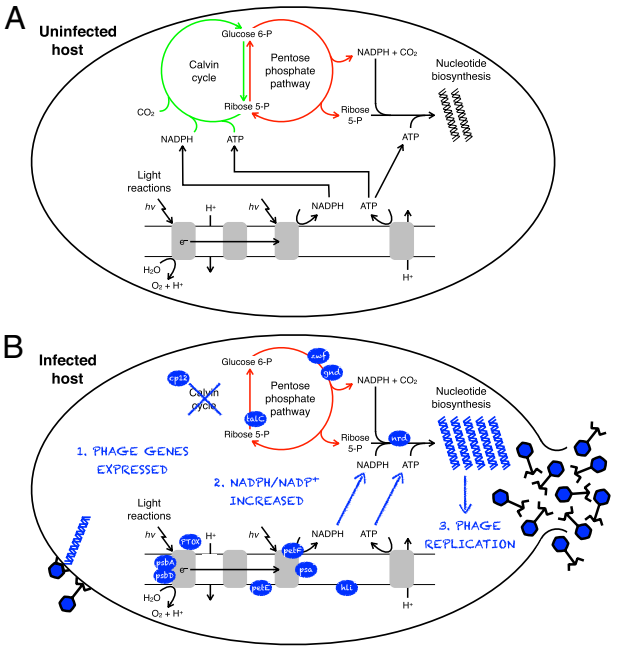

Fig. P1. Model of cyanobacterial metabolism during cyanophage infection. (*A*) In uninfected cells, four interrelated pathways combine to make DNA building blocks (nucleotides): the light reactions of photosynthesis, the Calvin cycle (the so-called "dark reactions" of photosynthesis), the pentose phosphate pathway, and nucleotide biosynthesis. (*B*) In infected cells, host-like genes (blue ovals) acquired by phage over evolutionary time are proposed to augment or inhibit key steps in host metabolism, leading to increased nucleotide biosynthesis for phage reproduction.

Author contributions: L.R.T., Q.Z., L.K., K.H.H., J.S., and S.W.C. designed research; L.R.T., Q.Z., L.K., K.H.H., and A.U.S. performed research; L.R.T., Q.Z., L.K., K.H.H., and A.U.S. analyzed data; and L.R.T., J.S., and S.W.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 3HJZ). The sequences reported in this paper have been deposited in the GenBank database [GU071107 (P-SSP2), GU071104 (P-HP1), and GU071102 (P-RSP5)].

[1]L.R.T. and Q.Z. contributed equally to this work.

[2]To whom correspondence may be addressed: chisholm@mit.edu or stubbe@mit.edu.

See full research article on page E757 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1102164108.

carbon from carbon dioxide, cyanobacteria are able to make sugar and transform it into nucleotides for replicating their genomes.

In the context of this simplified view of host metabolism, we noticed something peculiar about the collection of host-like genes found in cyanophage genomes: Genes for the light reactions and the pentose phosphate pathway were commonly found, as were those for nucleotide biosynthesis; however, no genes for the Calvin cycle were apparent, despite a search of more than 20 phage genomes. Although the presence of cyanophage photosynthesis proteins initially led to the proposal of increased carbon fixation in the sea (4), the absence of Calvin cycle genes and the presence of pentose phosphate genes suggested that an alternative model was required. Our model (Fig. P1*B*) suggests that NAPDH and ATP, the products of the light reactions, and redirection of carbon flux away from the Calvin cycle and through the pentose phosphate pathway to generate ribose 5-phosphate and NADPH, together would lead to enhanced nucleotide biosynthesis required for phage replication.

In support of this hypothesis was the discovery of a gene for a Calvin cycle inhibitor, CP12, in many phage genomes (5). CP12 is a host regulatory protein that binds to and inhibits two enzymes in the Calvin cycle, limiting carbon flux through this pathway and favoring flux through the pentose phosphate pathway. Thus, we propose that phages use the CP12 that they encode to shut down the Calvin cycle in the host, and redirect the products of the light reactions to their own ends.

Here we reveal that the *cp12* gene is widespread in both cultured and wild cyanophages, and is expressed during infection, concurrently with phage genes involved in the pentose phosphate pathway, genes for the light reactions of photosynthesis, and nucleotide biosynthesis. Thus, cyanophage infection appears to short-circuit host carbon metabolism, shutting down the Calvin cycle while boosting the three other pathways. As shown in Fig. P1*B*, cyanophages carry many genes (blue ovals) with putative functions in host metabolism. According to our model, the light reactions, aided by phage-encoded proteins, lead to increased production of NADPH and ATP. These storage molecules are not used to power carbon fixation because the Calvin cycle is blocked by phage-encoded CP12, forcing carbon flux through the pentose phosphate pathway, boosted by phage-encoded enzymes. NADPH, ATP, and ribose 5-phosphate produced by these processes are used to power phage nucleotide biosynthesis.

Several additional pieces of evidence support this model. NADPH, an electron carrier, is a product of the light reactions and the pentose phosphate pathway, and NADP is its electron-poor version. We show that, under infection, the ratio of NADPH to NADP increases. This result is compatible with increased activity of the light reactions and the pentose phosphate pathway for production of NADPH, the source of electrons for the synthesis of DNA building blocks. Furthermore, calculations imply that replicating cyanophages require significant de novo production of these nucleotides, and our model suggests how this might occur.

Finally, we studied the properties of the phage transaldolase enzyme, whose gene is the most widespread pentose phosphate pathway gene in cyanophages. The phage transaldolase is significantly shorter than the host transaldolase, and therefore we wondered why this enzyme would have been evolutionarily selected by phages over the host enzyme. We proposed that the phage transaldolase would have a higher activity than the host transaldolase. We therefore cloned, expressed, and purified several phage and host transaldolase enzymes, and measured their kinetic properties in vitro. We found that, to our surprise, the phage enzymes in fact had one third the kinetic efficiencies of the host enzymes. We therefore sought other explanations for phage use of this smaller enzyme. We hypothesized that the smaller size of the phage gene could be a significant benefit to the phage, with its small genome and limited resources from the host for replication. Indeed, we found that many phage genes are significantly shorter than their host orthologues, providing an evolutionary explanation for differences in gene sizes of shared phage–host orthologues in general.

The marine cyanobacteria–cyanophage system has proven to be an intriguing example of coevolution. In this study, we have attempted to tie the various evidentiary threads together to unveil the selective pressures that have led cyanophages to incorporate a particular set of host-like genes into their genomes. The key finding that cyanophages express the Calvin cycle inhibitor CP12 suggests that there are strong selective pressures for cyanophages to redirect host metabolism toward mobilizing energy, electrons, and carbon skeletons for DNA biosynthesis. Ultimate validation of this model will require a genetic system for phage and host, which is at present not available for these strains.

1. Millard A, Clokie MRJ, Shub DA, Mann NH (2004) Genetic organization of the *psbAD* region in phages infecting marine *Synechococcus* strains. *Proc Natl Acad Sci USA* 101: 11007–11012.
2. Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW (2005) Three *Prochlorococcus* cyanophage genomes: Signature features and ecological interpretations. *PLoS Biol* 3:e144.
3. Zinser ER, et al. (2009) Choreography of the transcriptome, photophysiology, and cell cycle of a minimal photoautotroph, *Prochlorococcus*. *PLoS ONE* 4:e5135.
4. Sharon I, et al. (2007) Viral photosynthetic reaction center genes and transcripts in the marine environment. *ISME J* 1:492–501.
5. Sullivan MB, et al. (2010) Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. *Environ Microbiol* 12:3035–3056 10.1111/j.1462–2920.2010.02280.x.