

## ORIGINAL ARTICLE

# Temporal transcriptomes of a marine cyanopodovirus and its *Synechococcus* host during infection

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**Abstract**

Marine picocyanobacteria belonging to genera *Synechococcus* and *Prochlorococcus* are genetically diverged and distributed into distinct biogeographical patterns, and both are infected by genetically closely related cyanopodoviruses. Previous studies have not fully explored whether the two virus–host systems share similar gene expression patterns during infection. Whole-genome expression dynamics of T7-like cyanopodovirus P-SSP7 and its host *Prochlorococcus* strain MED4 have already been reported. Here, we conducted genomic and transcriptomic analyses on T7-like cyanopodovirus S-SBP1 during its infection on *Synechococcus* strain WH7803. S-SBP1 has a latent period of 8 h and phage DNA production of 30 copies per cell. In terms of whole-genome phylogenetic relationships and average nucleotide identity, S-SBP1 was most similar to cyanopodovirus S-RIP2, which also infects *Synechococcus* WH7803. Three hyper-variable genomic islands were identified when comparing the genomes of S-SBP1 and S-RIP2. Single nucleotide variants were also observed in three S-SBP1 genes, which were located within the island regions. Based on RNA-seq analysis, S-SBP1 genes clustered into three temporal expression classes, whose gene content was similar to that of P-SSP7. Thirty-two host genes were upregulated during phage infection, including those involved in carbon metabolism, ribosome components, and stress response. These upregulated genes were similar to those upregulated by *Prochlorococcus* MED4 in response to infection by P-SSP7. Our study demonstrates a programmed temporal expression pattern of cyanopodoviruses and hosts during infection.

**KEYWORDS**

cyanophage genome, cyanopodovirus, RNA-seq, *Synechococcus*, transcriptome

## 1 | INTRODUCTION

Picocyanobacteria of the *Prochlorococcus* and *Synechococcus* genera are the most abundant primary producers in the global oceans, contributing up to 50% of the primary production in oligotrophic regions (Li, 1994; Liu et al., 1997). Cyanophages, viruses that infect cyanobacteria, play a great role in influencing the abundance, diversity, and evolution of their hosts (Avrani & Lindell, 2015; Mann, 2003; Suttle &

Chan, 1994). Most marine picocyanobacteria-infecting cyanophages recognized so far are lytic viruses that lead to host cell lysis. Almost all marine cyanophage isolates belong to one of three-tailed double-stranded DNA (dsDNA) virus families in the Caudovirales order: Myoviridae, Podoviridae, and Siphoviridae. Cyanopodoviruses and cyanomyoviruses are most frequently isolated from marine environments, while cyanosiphoviruses are rarely found (Sullivan et al., 2003; Suttle & Chan, 1993; Waterbury & Valois, 1993; Wilson et al., 1993).

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Metagenomic reads recruitment surveys also demonstrated that cyanopodoviruses and cyanomyoviruses are highly abundant in the Global Ocean (Huang et al., 2015b; Williamson et al., 2008), suggesting important ecological roles. Cyanophages also differ in host specificity. Cyanopodoviruses and cyanosiphoviruses commonly have a very narrow host range, while cyanomyoviruses have a much broader host range where some can infect both *Prochlorococcus* and *Synechococcus* strains (Sullivan et al., 2003; Wang & Chen, 2008).

The genomes of marine cyanopodoviruses are highly conserved in terms of genome architecture, gene content, and sequence similarity. All of the 20 genomes described are similar to the archetypical coliphage T7 (Chen & Lu, 2002; Huang et al., 2015a; Labrie et al., 2013; Pope et al., 2007; Sullivan et al., 2005). T7-like cyanopodoviruses vary little in genome size, which is usually around 45 kb. The genome can be divided into three consecutive modules that are respectively enriched with host take-over genes, replication genes, and virion structural genes. Through comparative genomic analysis, 15 core genes of the 20 cyanopodovirus genomes were determined, which constitute nearly half of each genome in length (Huang et al., 2015a). Cyanophages also encode several auxiliary metabolic genes (AMGs) involved in photosynthesis, carbon metabolism, the pentose phosphate pathway, and phosphorus acquisition (Lindell et al., 2004; Mann et al., 2003; Sullivan et al., 2005). Some of these genes, such as those encoding photosystem II D1 (*psbA*), high-light inducible protein (*hli*), and transaldolase (*talC*), are found in T7-like marine cyanopodoviruses (Huang et al., 2015a; Labrie et al., 2013; Sullivan et al., 2005). Interestingly, T7-like podoviruses such as cyanophages Pf-WMP3 (Liu et al., 2008), Pf-WMP4 (Liu et al., 2007), PP (Zhou et al., 2013), and A-4L (Ou et al., 2015) that infect freshwater cyanobacteria do not contain these genes.

The temporal transcriptional profiles of dsDNA phages are similarly programmed, where phage genes can be divided into three temporal classes of early, middle, and late expression (Guttman et al., 2005). Products of early genes allow the virus to redirect transcription from the host toward the phage. Proteins encoded by middle genes are involved in nucleotide metabolism and DNA replication. Meanwhile, late genes are responsible for viral particle formation, DNA packaging, and host cell lysis. Transcriptomic studies of cyanophages carried out using microarrays (Lindell et al., 2007) or RNA-seq (Doron et al., 2016; Lin et al., 2016; Thompson et al., 2016) have revealed that the T7-like *Prochlorococcus* phage P-SSP7 (Lindell et al., 2007) and the T4-like *Synechococcus* phage Syn9 (Doron et al., 2016) possess the typical three temporal modules of gene expression and that cyanophage AMGs are expressed during infection (Doron et al., 2016; Lin et al., 2016; Lindell et al., 2005, 2007; Thompson et al., 2016) and thought to be relevant for phage fitness (Zeng & Chisholm, 2012). A few host genes were found to be upregulated in response to phage infection, and these genes are often preferentially located in genomic islands of the host (Doron et al., 2016; Lindell et al., 2007).

*Prochlorococcus* and marine *Synechococcus* are considered as sister genera, which means they share a common ancestor. However, they are also phylogenetically divergent, have distinctive genomic

features, and occupy complementary though partly overlapping niches. Although the transcriptional process of the *Prochlorococcus* podovirus P-SSP7 and the response of its host have been reported (Lindell et al., 2007), it remains unclear whether *Synechococcus* and the podoviruses that infect them undergo similar transcriptional programs. In this study, we isolated a phage infecting *Synechococcus* strain WH7803 from Sanya Bay in the northern South China Sea. Morphological and genomic analyses indicated that the phage belongs to the T7-like cyanopodoviruses. Transcriptome analysis showed that this phage has a similar temporal gene expression profile to P-SSP7 and its *Synechococcus* host exhibits similar transcriptional responses as the *Prochlorococcus* host of P-SSP7.

## 2 | MATERIALS AND METHODS

### 2.1 | Phage isolation and propagation

The viral concentrate was prepared from 20 L of seawater collected in October 2014 from Sanya Bay in the South China Sea using a tangential flow filtration (TFF) system with a 30 kDa molecular weight cutoff filter (PALL Corporation). The host strain *Synechococcus* sp. WH7803 used to isolate phages was grown at 22°C in an A<sup>+</sup> artificial seawater medium under continuous cool white light with an intensity of 10  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Phages were isolated from viral concentrates using the double-layer agarose plate method. Plaques were screened by PCR using cyanopodovirus-specific primers targeting the DNA polymerase gene (*pol*) as previously described (Chen et al., 2009). After a plaque was identified to contain the gene *pol*, it was purified by three rounds of plaque assay and named S-SBP1. To obtain phage particles on a large scale, 500 ml phage lysate was concentrated using the PEG precipitation method. Ultrafiltration with Amicon<sup>®</sup> Ultra-15 30K Centrifugal Filter Units (Merck Millipore) was used to purify the concentrated phage particles. Five milliliters of phage concentrate in SM buffer (50 mM Tris-HCl, 100 mM NaCl, 8 mM MgSO<sub>4</sub>, 0.01% Gelatin) was obtained and stored at 4°C until further use.

### 2.2 | Transmission electron microscopy (TEM)

Viral particles in SM buffer were pipetted onto a 200-mesh Formvar-carbon-coated copper grid, stained with 2% phosphotungstic acid (pH = 7.0), and imaged using a JEM-100CX II transmission electron microscope (Akishima, Tokyo) at the Laboratory of Bio-Electron Microscopy, Sun Yat-sen University (Guangzhou, China).

### 2.3 | DNA extraction, genome sequencing, and annotation

Genomic DNA of the concentrated phage particles was extracted using the QIAamp MinElute Virus Spin Kit (Qiagen) according

to the manufacturer's instructions. The MiSeq PE300 platform (Illumina) was used to sequence the genome at Shanghai Majorbio Bio-Pharm Technology Co., Ltd. Clean reads were assembled by SOAP de novo v2.04. A single contig was generated after the assembly procedure. Chromosome end repeats were identified as previously described (Zhang et al., 2015). Briefly, the clean reads were mapped onto the assembled phage genome, and the terminal direct repeat was identified as the sequence fragment with a sharp increase of coverage comparing to adjacent regions. Open reading frames (ORFs) were predicted with GeneMarkS and tRNA-Scan SE was used to search for possible tRNA sequences. Genes were annotated by searching the translated sequences against the NCBI non-redundant (nr) protein database and the conserved domain database (CDD).

## 2.4 | Comparative genomic analysis

The complete genome of S-SBP1 was compared to 20 other cyanopodovirus genomes and the core genes were identified as previously described (Huang et al., 2015a). The concatenated amino acid sequences of core genes were aligned using ClustalX2, and the alignment was then trimmed using Gblocks. A phylogenetic tree was constructed using the neighbor-joining method and based on the trimmed alignment obtained with MEGA7. Average nucleotide identity (ANI) between genomes was calculated by using the program JSpecies v1.2.1. Mauve (version 20150226 build 10) was used for whole-genome alignment.

## 2.5 | Growth curve and transcriptomic experiment design

Exponentially growing host culture ( $4.7 \times 10^7$  cell  $\text{ml}^{-1}$ ) was inoculated with phage S-SBP1 at a multiplicity of infection (MOI) of 2. The phage lysate was titered using the plaque assay before adding to the host cultures. Host cell abundance was determined via flow

cytometry (BD Accuri™ C6; BD Biosciences) using the combination of side light scatter (SSC) and fluorescence from phycoerythrin (FL2, PE, 585/40 nm). To monitor phage growth, two aliquots of 1 ml culture were collected every 0.5–1 h during the first 16 h after adding the phage. One aliquot of the culture was centrifuged at  $13,680 \times g$  for 3 min to collect the supernatant (for measuring extracellular phage DNA concentration) while the other aliquot was not treated (for measuring total phage DNA concentration). The samples were flash-frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . At 15 min, 1 h, 3 h, 5 h, and 7 h after phage lysate addition, 100 ml of culture was sampled and centrifuged at  $7000 \times g$  for 10 min to collect cells for transcriptomic analysis. A parallel control group without phage addition was also sampled at the 30 min and 8 h time points, after which the pellet was flash-frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ .

Phage DNA concentration during cultivation was measured using real-time PCR (qPCR). Primers were designed to amplify a 187 bp fragment of S-SBP1 (forward: 5'-ATGGCTGTGGTCGGTCAAGAGG-3'; reverse: 5'-AGGCGGCTTGGTGTGTCAT-3'). qPCR was performed on the CFX Connect Real-Time System (Bio-Rad Laboratories) using a reaction mixture of 12.5  $\mu\text{l}$  SYBR Premix Ex Taq II (Tli RNaseH Plus; RR820A; Takara Bio), 1  $\mu\text{l}$  each of forward and reverse primers (10  $\mu\text{M}$ ), 1  $\mu\text{l}$  template, 1  $\mu\text{l}$  bovine serum albumin (BSA; Takara Bio), and 8.5  $\mu\text{l}$   $\text{H}_2\text{O}$ . The qPCR cycling conditions were as follows:  $95^\circ\text{C}$  for 30 s, 44 cycles of  $94^\circ\text{C}$  for 5 s,  $55^\circ\text{C}$  for 30 s, and  $72^\circ\text{C}$  for 45 s, followed by a melt curve step. Samples were heated at  $95^\circ\text{C}$  for 15 min to lyse the cells, and phage particles were then used as a qPCR template. A standard curve was prepared using 10-fold serial dilutions in  $\text{H}_2\text{O}$  of phage genomic DNA (gDNA). gDNA concentration was measured using the PicoGreen assay and the genome copy number was calculated as the DNA concentration/molecular weight of one genome chromosome. Linear regression was calculated between the logarithms of genome copy numbers and the Ct values ( $R^2 = 0.999$ , amplification efficiency = 91.75%). Phage DNA production was calculated according to the following formula,

$$\text{Phage DNA production} = \Delta V / \Delta B = (V_t - V_0) / (B_0 - B_t), \quad (1)$$

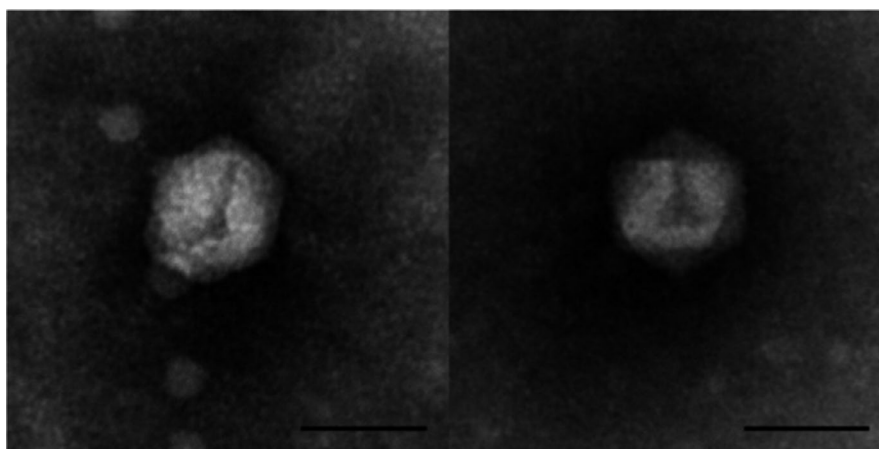
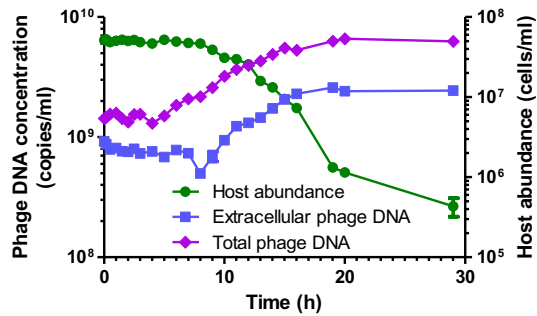


FIGURE 1 Transmission electronic microscopy micrograph of cyanopodovirus S-SBP1. Scale bar =100 nm



**FIGURE 2** Growth curve of cyanopodovirus S-SBP1. Phage DNA concentration was determined by quantifying the gene copy number using qPCR and host cell abundance by flow cytometry

where  $\Delta V$  represents the increase in extracellular phage DNA concentration measured by qPCR,  $\Delta B$  represents the reduction in host cell abundance determined by flow cytometry, and  $t = 19$  h.

## 2.6 | RNA-seq analysis

Total RNA was extracted using TRIzol Reagent (Invitrogen) and the RNeasy Mini Kit (Qiagen). Next-generation sequencing libraries were prepared using NEBNext Ultra™ Directional RNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer's instructions. Then, libraries with different indices were multiplexed and loaded on an Illumina HiSeq instrument according to the manufacturer's instructions. Sequencing was carried out using a  $2 \times 150$  paired-end (PE) configuration, followed by processing and analysis by GENEWIZ.

Cutadapt (v1.9.1) was used to remove adapters, primers, and reads with a base quality  $<20$  based on FASTQ files. Clean data were aligned to the S-SBP1 and WH7803 genomes via Bowtie2 software (v2.1.0). HTSeq (v0.6.1p1) was used to estimate gene expression levels from the PE clean data. Differential expression analysis was performed using the DESeq Bioconductor package, a model based on the negative binomial distribution. After adjusting using Benjamini and Hochberg's approach for controlling the false discovery rate,

differentially expressed genes were considered significant at  $p$  values  $<0.05$ . Samtools v0.1.19 and Bcftools v0.1.19+ were used to carry out a single nucleotide variant (SNV) calling.

To cluster phage genes based on their transcriptional patterns, the number of reads mapped to each S-SBP1 gene was normalized to its gene size, and then transcript levels of a particular gene across the five-time points were converted to a range from 0 (minimum expression) to 1 (maximum expression). Clustering was performed using the Bray-Curtis similarity and group average linkage in PRIMER 5 software. The EdgeR package in Bioconductor was used to analyze the differential expression of host genes. As no genes of significantly different transcript levels were found between the two control samples (i.e., no phage was added; collected at 30 min and 8 h), phage-treated samples were only compared to the control sample collected at 30 min. Host genes with  $\log_2 FC \geq 2$  as well as  $p < 0.05$  were considered to be upregulated.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Phage isolation and characterization

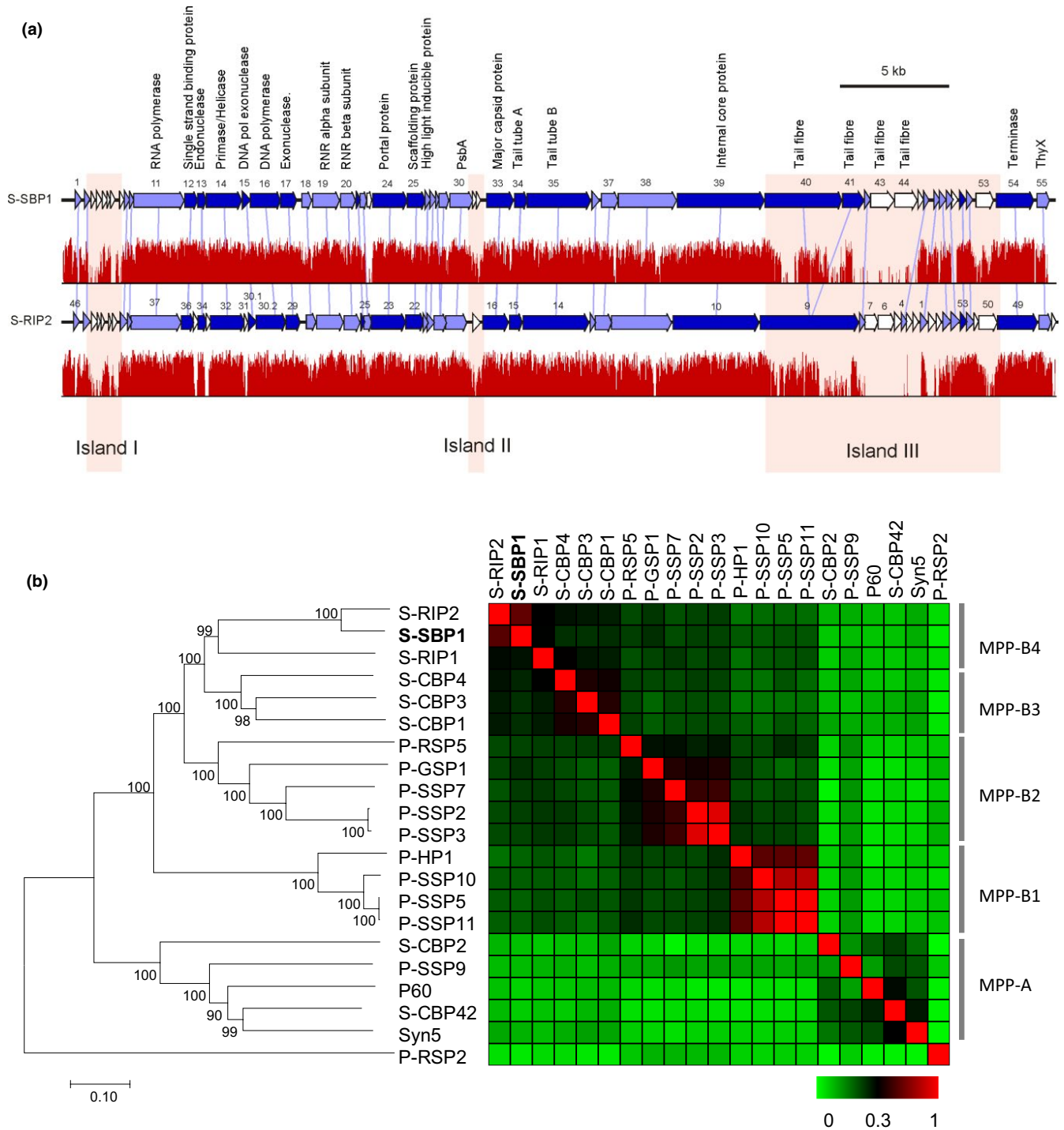
A bacteriophage that infects the marine *Synechococcus* strain WH7803 was isolated from seawater collected from Sanya Bay in the South China Sea using the double-layer agarose plating method. A round clear plaque was observed on the plate and host cells growing in liquid A<sup>+</sup> medium were lysed, indicating that the isolated bacteriophage is a lytic phage. TEM micrographs showed that this phage has an icosahedral head ( $51.26 \pm 0.61$  nm in diameter) (Figure 1) We did not observe a clear tail on the micrographs obtained, while the genome of this phage showed clear similarity to T7-like cyanopodoviruses (see below) which generally have a short tail. As the phage exhibits genomic features of podoviruses, we named it S-SBP1, where S stands for *Synechococcus*, SB for Sanya Bay, and P for podovirus.

To monitor phage growth, we used qPCR to measure the extracellular and total (extracellular and intracellular) phage DNA

**TABLE 1** Latent period and phage production of seven marine cyanopodoviruses

Phage	Group	Host	Latent period (h)	Phage production	Reference
P60	MPP-A	<i>Synechococcus</i> WH7805	1.5	80	Chen and Lu (2002), Wang and Chen (2008)
Syn5	MPP-A	<i>Synechococcus</i> WH8019	1	30	Pope et al. (2007), Raytcheva et al. (2011)
S-CBP2	MPP-A	<i>Synechococcus</i> CB0208	8–10	92	Wang and Chen (2008)
P-SSP7	MPP-B	<i>Prochlorococcus</i> MED4	8	40	Frois-Moniz (2014), Lindell et al. (2005)
S-CBP1	MPP-B	<i>Synechococcus</i> CB0101	6–8	86	Wang and Chen (2008)
S-CBP3	MPP-B	<i>Synechococcus</i> CB0101	8–10	75	Wang and Chen (2008)
S-SBP1	MPP-B	<i>Synechococcus</i> WH7803	8	33	This study

*Note:* Phage production of isolates was measured via different methods in the various studies, such as measuring plaque-forming unit (PFU) for Raytcheva et al. (2011), total viral-like particles (VLPs) for Wang and Chen (2008), and phage DNA production via qPCR for Frois-Moniz (2014) and this study.



**FIGURE 3** Genome map of cyanopodoviruses S-SBP1 and its relationship to other marine cyanopodoviruses. (a) Genome map of S-SBP1 and S-RIP2. Arrows represent predicted genes (dark blue, core genes shared by the 21 cyanopodovirus genomes; light blue, other homologs between S-SBP1 and S-RIP2; white, unique genes of S-SBP1 or S-RIP2). Red bars indicate the genomic similarity generated after aligning the two genomes using Mauve. Potential genomic islands are shaded in orange. (b) A whole-genome phylogenetic tree was built based on the alignment of concatenated amino acid sequences of the 15 core genes shared by all 21 cyanopodovirus genomes. The map shows the average nucleotide identities between any pair of the 21 cyanopodovirus genomes

concentration. The growth curve showed that the phage starts to replicate its DNA 4 h after adding phage lysate to the host culture and starts to release phage progeny after nearly 8 h (Figure 2). This indicates that the phage has a latent period of ~8 h. We calculated the production of phage DNA by dividing the decrease in host cell

abundance by the increase in extracellular phage abundance. Using this method, the phage DNA production was determined to be ~33 copies per cell (Table 1).

Cyanopodoviruses are frequently isolated from marine environments (Sullivan et al., 2003; Suttle & Chan, 1993; Wang & Chen,

TABLE 2 Single nucleotide variants in the transcriptome of phage S-SBP1

Genomic island	Gene no.	Position	Reference	Alternative	SNV	Change in amino acid
Genomic island 1	9	3163	A	G	GCA>GCG	A>A
		3164	C	G	CTT>GTT	L>V
Genomic island 3	47	40076	G	C	GGC>GCC	G>A
Genomic island 3	50	41091	A	G	ATG>GTA	M>V
		41093	G	A		

2008; Waterbury & Valois, 1993); however, they are not all well-characterized physiologically, leading to a lack of information on their virological features, such as latent period and burst size. In addition to S-SBP1 in this study, six marine cyanopodoviruses, including *Synechococcus* phages P60 (Chen & Lu, 2002; Wang & Chen, 2008), Syn5 (Pope et al., 2007; Raytcheva et al., 2011), S-CBP1 (Wang & Chen, 2008), S-CBP2 (Wang & Chen, 2008), S-CBP3 (Wang & Chen, 2008), and *Prochlorococcus* phage P-SSP7 (Frois-Moniz, 2014; Lindell et al., 2005), have been characterized; their phage production and latent period are listed in Table 1. Note that, burst size, which is the number of infective progeny phages produced by a single infected host cell, was measured via different methods in the various studies, such as measuring plaque-forming units (PFU), viral-like particles (VLPs), and phage gene copies (see the footnote of Table 1). As measuring VLPs and gene copies does not provide exact phage burst size, we used “phage production” rather than “burst size” here. These data indicated that a few members of marine cyanopodoviruses have similar latent periods (e.g., ~1 or 8 h) or phage productions (e.g., ~30 or 80 per cell), although these parameters were overall highly variable among marine cyanopodovirus isolates. In contrast to burst size, which is difficult to measure accurately (Kirzner et al., 2016), the latent period was easier to determine. P60 and Syn5, two *Synechococcus* podoviruses within the MPP-A phylogenetic group (see Figure 3b and description below), have much shorter latent periods than those of the other five phage isolates. It would be interesting to further investigate the widely varying infection kinetics of cyanopodoviruses.

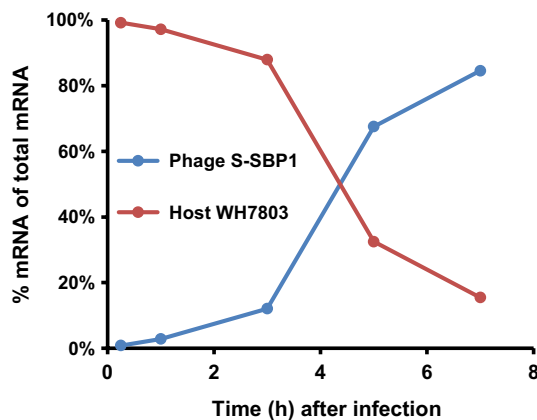
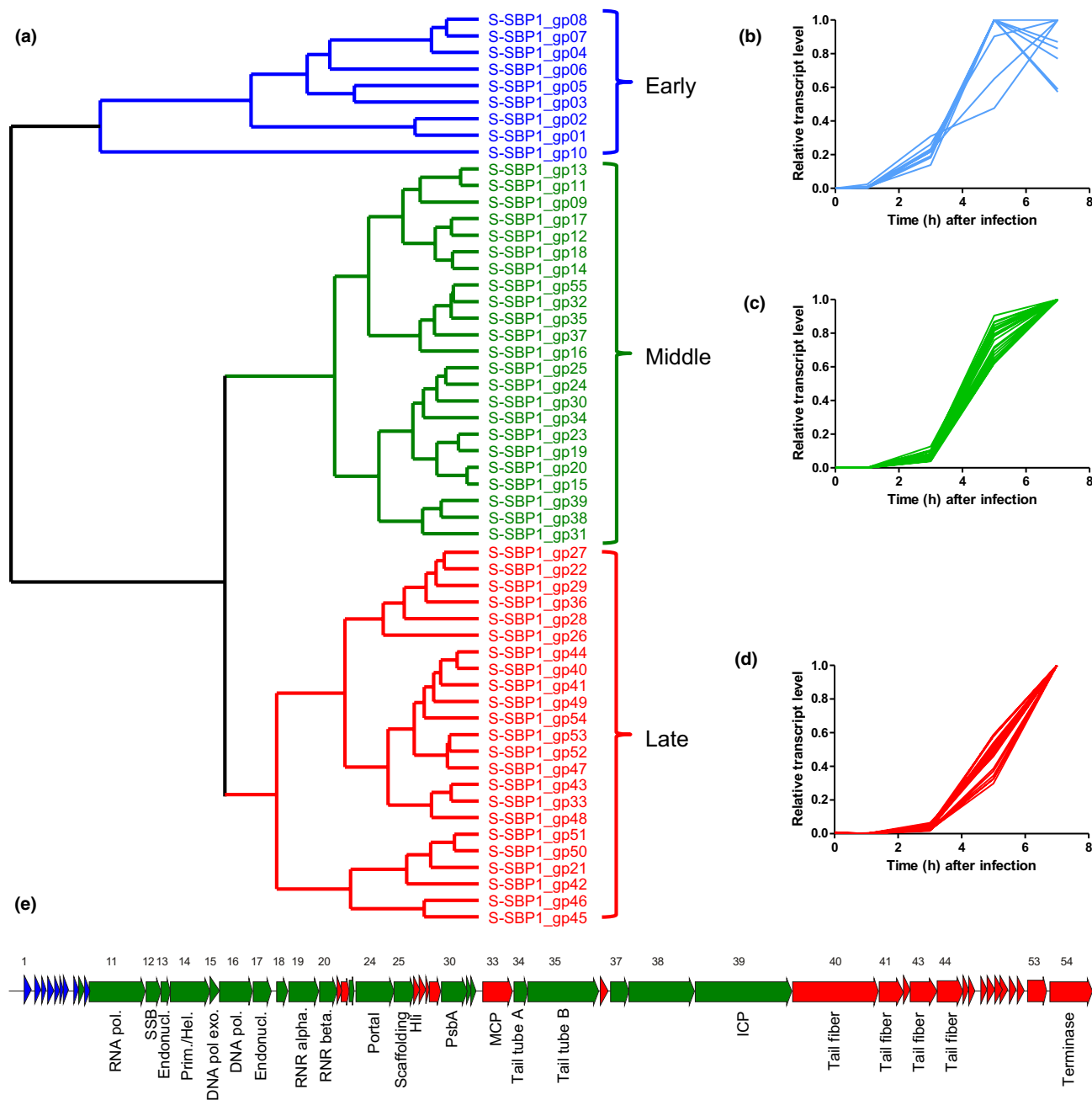


FIGURE 4 Ratios of phage and host mRNA. The ratios were determined from the RNA-seq reads mapped to phage S-SBP1 and host WH7803 genomes

### 3.2 | Phage genome and comparative genomic analysis

The complete genome of S-SBP1 is 45,519 bp in length with a GC content of 46.8%. During the assembly procedure, we found a 180 bp DNA fragment with higher coverage than that of its adjacent sequences (see Figure A1). This indicates that this phage genome has 180 bp direct terminal repeats at both chromosome ends (Zhang et al., 2015), which is a typical genome feature of T7-like phages including T7-like cyanopodoviruses (Sabehi & Lindell, 2012). A total of 55 ORFs were predicted, most of which (42 out of 55) were homologous to genes of *Synechococcus* podovirus S-RIP2 (GenBank accession: HQ317389), which was isolated from the Rhode Island Sound (Figure 3a). Moreover, S-SBP1 contains the 15 core genes that are shared by all 20 marine cyanopodoviruses (Huang et al., 2015a). A whole-genome phylogenetic tree based on the concatenated core genes showed that S-SBP1 was most closely related to S-RIP2 (Figure 3b). Moreover, among the 21 cyanopodovirus genomes analyzed, S-SBP1 and S-RIP2 shared the highest ANI (55%; Figure 3b). A heat map illustrating the pairwise ANI pattern of the 21 genomes corresponded to the phylogenetic clustering pattern, with more phylogenetically related phages having higher ANI values (Figure 3b).

The gene content and arrangement of S-SBP1 were also highly similar to S-RIP2. After comparing the whole genomes via BLASTP, S-SBP1 was found to have 14 unique genes (i.e., without homologs in S-RIP2) while S-RIP2 has 16 (Figure 3a). Most of these unique genes were located in three genomic islands; island I contained 5–6 small genes without recognized function that were located in the left extremity of the genome. Island II was adjacent to the *psbA* gene and contained two genes, while island III encompassed four genes coding for tail fibers and several small genes of unknown function. These island regions corresponded to the three genomic islands previously defined based on comparative genomic analysis of 12 cyanopodovirus genomes (Labrie et al., 2013). SNVs were found in three genes after performing SNV calling with the transcriptome sequencing data (Table 2). Interestingly, these genes, located in the genomic island I or III, were small and encoded proteins ( $\leq 100$  amino acids) with unknown functions. Our data are consistent with a previous study where mutations were found enriched in metagenomic islands of *Prochlorococcus* podoviruses (Schwartz & Lindell, 2017). Genomic islands unveiled through comparative genomic analysis are largely in agreement with those identified through metagenomic fragment



**FIGURE 5** Temporal transcriptional profiles of cyanopodovirus S-SBP1. (a) Genes were clustered based on their expression profiles during the infection period. Early, middle, and late expression clusters are shown in blue, green, and red, respectively. (b–d) The expression dynamics of individual genes in each expression cluster. (e) A genome map illustrating the temporal expression of each gene. Genes in the early, middle and late expression clusters are colored in blue, green, and red, respectively

recruitment (Labrie et al., 2013; Schwartz & Lindell, 2017). Likely, mutations occur more frequently in phage genomic island regions, which may be hot spots responsible for phage genome diversity.

### 3.3 | Transcriptomic analysis

To investigate the transcriptome dynamics of phage S-SBP1 during infection of its host *Synechococcus* WH7803, samples at 15 min, 1 h,

3 h, 5 h, and 7 h after infection were subjected to RNA-seq analysis. During the 8 h latent period, phage transcription took over the host transcription at the 5 h time point, with phage mRNA constituting 67% of the total mRNA (Figure 4). This is consistent with the observation that intracellular phage DNA began to accumulate at 4 h post-infection (Figure 2).

The phage genes were clustered into three classes based on their temporal expression pattern (Figure 5a). The early expression class contained nine genes (Figure 5b), which were located in

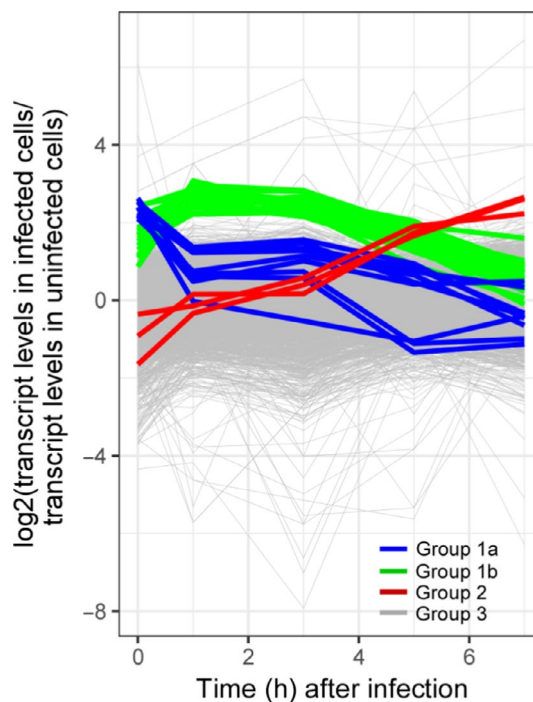
the left end of the genome (Figure 5e) and were small in size; thus, these genes were considered to be associated with host take-over. The middle expression class contained 23 genes (Figure 5c), most of which were involved in DNA replication, while five were associated with phage particle formation and coded for phage tail tube proteins and internal core proteins. The thymidylate synthase gene *thyX* located at the right end of the genome map was also clustered with the middle class. Finally, the late expression class consisted of 23 genes (Figure 5d), 15 of which were located in the right arm of the genome and are involved in phage tail formation. The other eight late genes were not arranged together on the genome map (Figure 5e).

Transcriptome profiling conducted throughout infection of S-SBP1 showed similar data to that of the podovirus P-SSP7 that infects *Prochlorococcus* strain MED4 (Lindell et al., 2007). However, in contrast to the exact linear arrangement of the early (genes 1–12), middle (genes 13–28), and late (genes 29–51) expression clusters of P-SSP7 (Lindell et al., 2007), eight late genes were located in the middle region of S-SBP1 genome (Figure 5e). Moreover, five phage structural genes (genes 34, 35, 37, 38, and 39) belonged to the middle class of S-SBP1, while their homologous genes in P-SSP7 were within the late class (Lindell et al., 2007). The middle class of P-SSP7 contains four “bacterial-like” genes: the ribonucleotide reductase gene *nrd*, the high-light-inducible gene *hli*, the photosystem II gene *psbA*, and the transaldolase gene *talC* (Lindell et al., 2007). Interestingly, the *thyX* gene, which is located at the right end of the S-SBP1 genome, corresponding to the *talC* locus in P-SSP7 (Sullivan et al., 2005), also belonged to the middle class (Lindell et al., 2007). Moreover, at this locus, *Synechococcus* podoviruses contain the *thyX* gene while *Prochlorococcus* podoviruses contain the *talC* gene (Huang et al., 2015a). The cyanophage transaldolase protein may be able to redirect the host metabolism toward generating precursors and ribose substrates for phage nucleotide synthesis (Lindell et al., 2007; Thompson et al., 2011; Zeng & Chisholm, 2012). Thymidylate synthase functions in the synthesis of the essential DNA precursor, thymidylate (dTMP) (Myllykallio et al., 2002). Thus, both *talC* and *thyX* genes are thought to facilitate the biosynthesis of deoxynucleotides for phage DNA replication (Huang et al., 2015a). Our results suggest that these two functionally different genes located in the same phage genome locus have a similar transcriptional time course, playing analogous roles in providing energy or substrates for phage replication.

The response of the host transcriptome during infection was analyzed by comparing the transcript levels between infected and uninfected hosts. A total of 32 host genes were significantly upregulated, while other genes were downregulated or remained unaltered (Figure 6, Table 3). The upregulated genes were classified into three groups. Group 1a consisted of nine genes, such as *ccmK*, *rbcl*, *rbcs*, and *csoS*, which were immediately upregulated after phage addition and appear to be associated with carbon metabolism. Genes of group 1b code for ribosomal proteins and were upregulated within 1–3 h after phage addition. Together, groups 1a and 1b consist of genes that were upregulated during the early period of infection. Group 2 contains three genes, including *umuC*, *umuD*, and one unknown gene, that were

upregulated during the late period of infection (5–7 h after phage addition) and putatively associated with stress responses.

The transcriptional response of the *Synechococcus* host of phage S-SBP1 was also similar to that of the *Prochlorococcus* host of phage P-SSP7 during infection (Lindell et al., 2007). First, only a few host genes (32 in *Synechococcus* and 41 in *Prochlorococcus*) were upregulated in both host strains. Second, there was a similar content of upregulated genes in the two hosts, such as genes associated with carbon metabolism (*rbcl*, *rbcs*, and *csoS*) and ribosome components (*rpl* and *rps*) during the early period of infection, and genes associated with stress responses (*umuD*) during the late period of infection. However, a number of significantly upregulated genes in the *Prochlorococcus* host, such as those involved in transcription (*rpoC2*, *rpoD*), RNA degradation and modification (*rne*, *rnhB*, *dus*, and *sun*), protein turnover (*clpS*), and stress responses (*hli*, *phoH*) (Lindell et al., 2007) were not upregulated in the *Synechococcus* host. Many of these genes are located in the hypervariable genomic islands of the *Prochlorococcus* host strain (Lindell et al., 2007). Therefore, it is likely that some of the upregulated genes are not universal in picocyanobacterial genomes and that genes upregulated in response to phage infection are host strain-specific. This can explain why the upregulated expression of several host genes was not commonly observed in both cyanobacteria strains after phage infection. This is also evident when different *Synechococcus* host strains are infected with cyanomyoviruses that exhibit a broad host range (Doron et al., 2016).



**FIGURE 6** Transcriptional profiles of *Synechococcus* WH7803 genes during infection with S-SBP1. The transcript levels are presented as  $\log_2$ -fold change in infected cells relative to uninfected cells. Group 1a + 1b and 2 include genes that were upregulated within 1–3 h and 5–7 h after infection, respectively. Group 3 includes genes whose expression remained unchanged or decreased



TABLE 3 Upregulated genes of *Synechococcus* WH7803 during infection with podovirus S-SBP1

Group	ORF	Gene product	Log <sub>2</sub> FC, fold change (infected/uninfected)				
			15 min	1 h	3 h	5 h	7 h
Group 1a	SYNWH7803_RS03420	Carbon dioxide-concentrating mechanism protein, <i>ccmK</i>	2.25 <sup>*</sup>	1.23	1.28	0.74	0.34
	SYNWH7803_RS03425	Ribulose biphosphate carboxylase large subunit, <i>rbcl</i>	2.53 <sup>*</sup>	1.37 <sup>*</sup>	1.56 <sup>*</sup>	0.94	-0.46
	SYNWH7803_RS03430	Ribulose biphosphate carboxylase small subunit, <i>rbcS</i>	2.11 <sup>*</sup>	0.75	1.14	0.65	-0.64
	SYNWH7803_RS03435	Carboxysome shell protein, <i>csoS</i>	2.26 <sup>*</sup>	1.32 <sup>*</sup>	1.34 <sup>*</sup>	0.84	-0.33
	SYNWH7803_RS04285	Alpha/beta hydrolase	2.19 <sup>*</sup>	0.49	1.01	0.42	0.49
	SYNWH7803_RS04795	Hypothetical protein	2.40 <sup>*</sup>	-0.03	-0.53	-1.05	-0.40
	SYNWH7803_RS05510	Peroxiredoxin	2.10 <sup>*</sup>	1.37 <sup>*</sup>	1.47 <sup>*</sup>	0.47	-0.39
	SYNWH7803_RS10040	DUF4278 domain-containing protein	2.63 <sup>*</sup>	0.62	0.57	-1.34	-1.13
	SYNWH7803_RS10045	Hypothetical protein	2.55 <sup>*</sup>	0.68	0.74	-1.11	-0.99
Group 1b	SYNWH7803_RS02060	50S ribosomal protein L15	1.10	2.47 <sup>*</sup>	2.30 <sup>*</sup>	1.31	0.60
	SYNWH7803_RS02065	30S ribosomal protein S5	1.15	2.59 <sup>*</sup>	2.46 <sup>*</sup>	1.83 <sup>*</sup>	0.83
	SYNWH7803_RS02070	50S ribosomal protein L18	1.30	2.50 <sup>*</sup>	2.68 <sup>*</sup>	1.46 <sup>*</sup>	1.03
	SYNWH7803_RS02075	50S ribosomal protein L6	1.78 <sup>*</sup>	2.81 <sup>*</sup>	2.82 <sup>*</sup>	1.81 <sup>*</sup>	0.83
	SYNWH7803_RS02080	30S ribosomal protein S8	1.52 <sup>*</sup>	2.80 <sup>*</sup>	2.59 <sup>*</sup>	2.05 <sup>*</sup>	0.70
	SYNWH7803_RS02085	50S ribosomal protein L5	1.70 <sup>*</sup>	2.97 <sup>*</sup>	2.83 <sup>*</sup>	1.87 <sup>*</sup>	0.54
	SYNWH7803_RS02090	50S ribosomal protein L24	1.87 <sup>*</sup>	2.83 <sup>*</sup>	2.57 <sup>*</sup>	1.96 <sup>*</sup>	0.93
	SYNWH7803_RS02095	50S ribosomal protein L14	1.87 <sup>*</sup>	2.85 <sup>*</sup>	2.48 <sup>*</sup>	1.61 <sup>*</sup>	0.22
	SYNWH7803_RS02100	30S ribosomal protein S17	1.81 <sup>*</sup>	2.68 <sup>*</sup>	2.42 <sup>*</sup>	1.35	-0.11
	SYNWH7803_RS02110	50S ribosomal protein L16	1.40 <sup>*</sup>	2.39 <sup>*</sup>	2.28 <sup>*</sup>	1.67 <sup>*</sup>	0.75
	SYNWH7803_RS02115	30S ribosomal protein S3	1.67 <sup>*</sup>	2.80 <sup>*</sup>	2.51 <sup>*</sup>	1.52 <sup>*</sup>	0.41
	SYNWH7803_RS02120	50S ribosomal protein L22	2.41 <sup>*</sup>	2.88 <sup>*</sup>	2.81 <sup>*</sup>	1.58 <sup>*</sup>	0.88
	SYNWH7803_RS02130	50S ribosomal protein L2	2.15 <sup>*</sup>	2.75 <sup>*</sup>	2.56 <sup>*</sup>	1.42 <sup>*</sup>	0.45
	SYNWH7803_RS02135	50S ribosomal protein L23	1.83 <sup>*</sup>	2.22 <sup>*</sup>	2.27 <sup>*</sup>	0.90	0.05
	SYNWH7803_RS02140	50S ribosomal protein L4	2.45 <sup>*</sup>	2.70 <sup>*</sup>	2.47 <sup>*</sup>	1.45	0.72
	SYNWH7803_RS02145	50S ribosomal protein L3	1.87 <sup>*</sup>	2.47 <sup>*</sup>	2.15 <sup>*</sup>	1.27	0.25
	SYNWH7803_RS02995	30S ribosomal protein S4	1.42 <sup>*</sup>	2.23 <sup>*</sup>	2.46 <sup>*</sup>	1.25	0.31
	SYNWH7803_RS11725	50S ribosomal protein L7/L12	0.87	2.96 <sup>*</sup>	2.30 <sup>*</sup>	1.71 <sup>*</sup>	1.02
	SYNWH7803_RS11730	50S ribosomal protein L10	0.87	3.07 <sup>*</sup>	2.43 <sup>*</sup>	1.93 <sup>*</sup>	1.60
	SYNWH7803_RS11735	50S ribosomal protein L1	1.60 <sup>*</sup>	2.37 <sup>*</sup>	2.63 <sup>*</sup>	1.29	0.32
Group 2	SYNWH7803_RS00835	Hypothetical protein	-0.92	0.15	0.17	1.73 <sup>*</sup>	2.66 <sup>*</sup>
	SYNWH7803_RS05320	Nucleotidyltransferase, <i>umuC</i>	-1.65	-0.33	0.39	1.66 <sup>*</sup>	2.62 <sup>*</sup>
	SYNWH7803_RS05325	SOS-regulated protein, <i>umuD</i>	-0.36	-0.14	0.58	1.90 <sup>*</sup>	2.23 <sup>*</sup>

Note: Upregulated gene meets the criterion: log<sub>2</sub>FC ≥ 2 and *p* < 0.05.

Abbreviation: ORF, open reading frame.

\**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

## 4 | CONCLUSIONS

T7-like cyanopodoviruses that infect marine picocyanobacterial *Prochlorococcus* and *Synechococcus* form a relatively genetically conserved phage regime. Marine cyanopodoviruses seem to employ a programmed transcriptional strategy to process infection and the two different host types also respond similarly to infection. Despite

the genomic conservation of marine T7-like cyanopodoviruses, hot spots for genetic diversity exist in specific genome regions, leading to the occurrence of genomic islands. Genes within these genomic islands are likely involved in the interaction with hosts during infection, such as host take-over genes that are expressed during the early infection period and phage tail-related genes, which are predicted to be responsible for host recognition and are expressed during the late

infection cycle. Our study highlights the genomic and transcriptomic conservation of marine cyanopodoviruses, which may help to maintain a stable virus-host relationship. Future studies should explore the diverged genes enriched in phage genomic islands and their role on the transcriptome during the virus-host interaction.

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

**Sijun Huang:** Conceptualization (lead); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (lead); Project administration (lead); Writing-original draft (lead); Writing-review & editing (lead). **Yingting Sun:** Data curation (equal); Formal analysis (equal); Investigation (equal). **Si Zhang:** Resources (equal); Supervision (equal). **Lijuan Long:** Funding acquisition (equal); Resources (equal); Supervision (equal).

## ETHICS STATEMENT

None required.

## DATA AVAILABILITY STATEMENT

The complete genome sequence of cyanophage S-SBP1 has been deposited in GenBank under the accession number MT424636: <https://www.ncbi.nlm.nih.gov/nuccore/MT424636>. Transcriptomic data have been deposited in GEO under the accession number GSE150732: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150732>.

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## APPENDIX

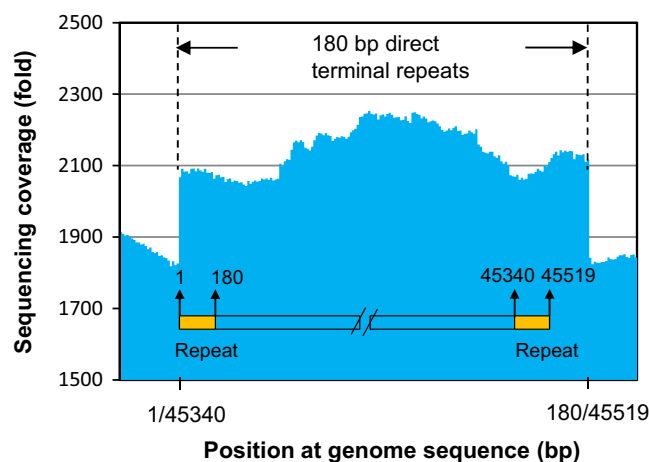


Figure A1 High sequencing coverage at the direct terminal repeats of the whole genome of *Synechococcus* phage S-SBP1