
Article:
Rihtman, B., Bowman-Grahl, S., Millard, A. et al. (3 more authors) (2019) Cyanophage MazG is a pyrophosphohydrolase but unable to hydrolyse magic spot nucleotides. Environmental Microbiology Reports, 11 (3). pp. 448-455. ISSN 1758-2229

https://doi.org/10.1111/1758-2229.12741

---

environmental microbiology reports

Environmental Microbiology Reports (2019) **11**(3), 448–455 doi:10.1111/1758-2229.12741

Brief Report

Cyanophage MazG is a pyrophosphohydrolase but unable to hydrolyse magic spot nucleotides

Branko Rihtman,[1] Sabine Bowman-Grahl,[1#] Andrew Millard,[2] Rebecca M. Corrigan,[3] Martha R. J. Clokie[2] and David J. Scanlan[1*]

[1]*School of Life Sciences, University of Warwick, Coventry, UK.*

[2]*Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK.*

[3]*Department of Molecular Biology & Biotechnology, University of Sheffield, Sheffield, UK.*

Summary

Bacteriophage possess a variety of auxiliary metabolic genes of bacterial origin. These proteins enable them to maximize infection efficiency, subverting bacterial metabolic processes for the purpose of viral genome replication and synthesis of the next generation of virion progeny. Here, we examined the enzymatic activity of a cyanophage MazG protein – a putative pyrophosphohydrolase previously implicated in regulation of the stringent response via reducing levels of the central alarmone molecule (p)ppGpp. We demonstrate, however, that the purified viral MazG shows no binding or hydrolysis activity against (p)ppGpp. Instead, dGTP and dCTP appear to be the preferred substrates of this protein, consistent with a role preferentially hydrolysing deoxyribonucleotides from the high GC content host *Synechococcus* genome. This showcases a new example of the fine-tuned nature of viral metabolic processes.

Introduction

Cyanophage that infect the marine cyanobacterial genera *Synechococcus* and *Prochlorococcus* are widespread and abundant in oceanic systems (Suttle and Chan, 1994; Sullivan *et al.,* 2003; Baran *et al.,* 2018) where they play important ecosystem roles including releasing organic matter through cell lysis (Suttle, 2007), transferring genes horizontally between hosts (Zeidner *et al.,* 2005) and structuring host communities (Mühling *et al.,* 2005). Cyanophage can also influence ocean biogeochemistry by modifying host metabolism during the infection process, such as the shutdown of CO_2 fixation whilst maintaining photosynthetic electron transport (Puxty *et al.,* 2016). This subversion of host metabolism is facilitated by the expression of cyanophage genes that appear to have a bacterial origin, so-called auxiliary metabolic genes (AMGs) (Breitbart *et al.,* 2007). These include genes involved in photosynthesis (Mann *et al.,* 2003; Lindell *et al.,* 2005; Fridman *et al.,* 2017) and photoprotection (Lindell *et al.,* 2004; Millard *et al.,* 2004; Sullivan *et al.,* 2005; Roitman *et al.,* 2018), pigment biosynthesis (Dammeyer *et al.,* 2008), central carbon metabolism (Millard *et al.,* 2009; Thompson *et al.,* 2011), nucleotide biosynthesis (Enav *et al.,* 2014), phosphorus metabolism (Sullivan *et al.,* 2010; Zeng and Chisholm, 2012; Lin *et al.,* 2016) and other stress responses (Sullivan *et al.,* 2010; Crummett *et al.,* 2016).

Amongst the cyanophage AMGs MazG is a core gene in cyanomyoviruses (Millard *et al.,* 2009; Sullivan *et al.,* 2010) and of particular interest since it has been proposed to play a more general role in regulating host metabolism (Clokie and Mann, 2006; Clokie *et al.,* 2010). In *Escherichia coli,* MazG has been implicated in regulating programmed cell death by interfering with the function of the MazEF toxin-antitoxin system, through lowering of cellular (p)ppGpp levels (Gross *et al.,* 2006). This latter molecule guanosine 3′,5′ bispyrophosphate, together with guanosine pentaphosphate also known as magic spot nucleotides, is a global regulator of gene expression in bacteria (Traxler *et al.,* 2008) synthesized by RelA under amino acid starvation. Since MazG can potentially regulate levels of (p)ppGpp in *E. coli,* a similar role has been proposed for the cyanophage encoded MazG (Clokie and Mann, 2006). This is pertinent given that picocyanobacterial hosts like *Synechococcus* and *Prochlorococcus* occupy oligotrophic conditions (see Scanlan *et al.,* 2009; Biller *et al.,* 2015) where nutrient starvation is likely and (p)ppGpp may be involved in adapting to this stressed state. By regulating (p)ppGpp levels the cyanophage encoded MazG

*For correspondence. E-mail d.j.scanlan@warwick.ac.uk; Tel. (+44) 24 76 528363; Fax (+44) 24 76 523701. #Present address: Immunocore Ltd, Milton Park, Abingdon, UK.

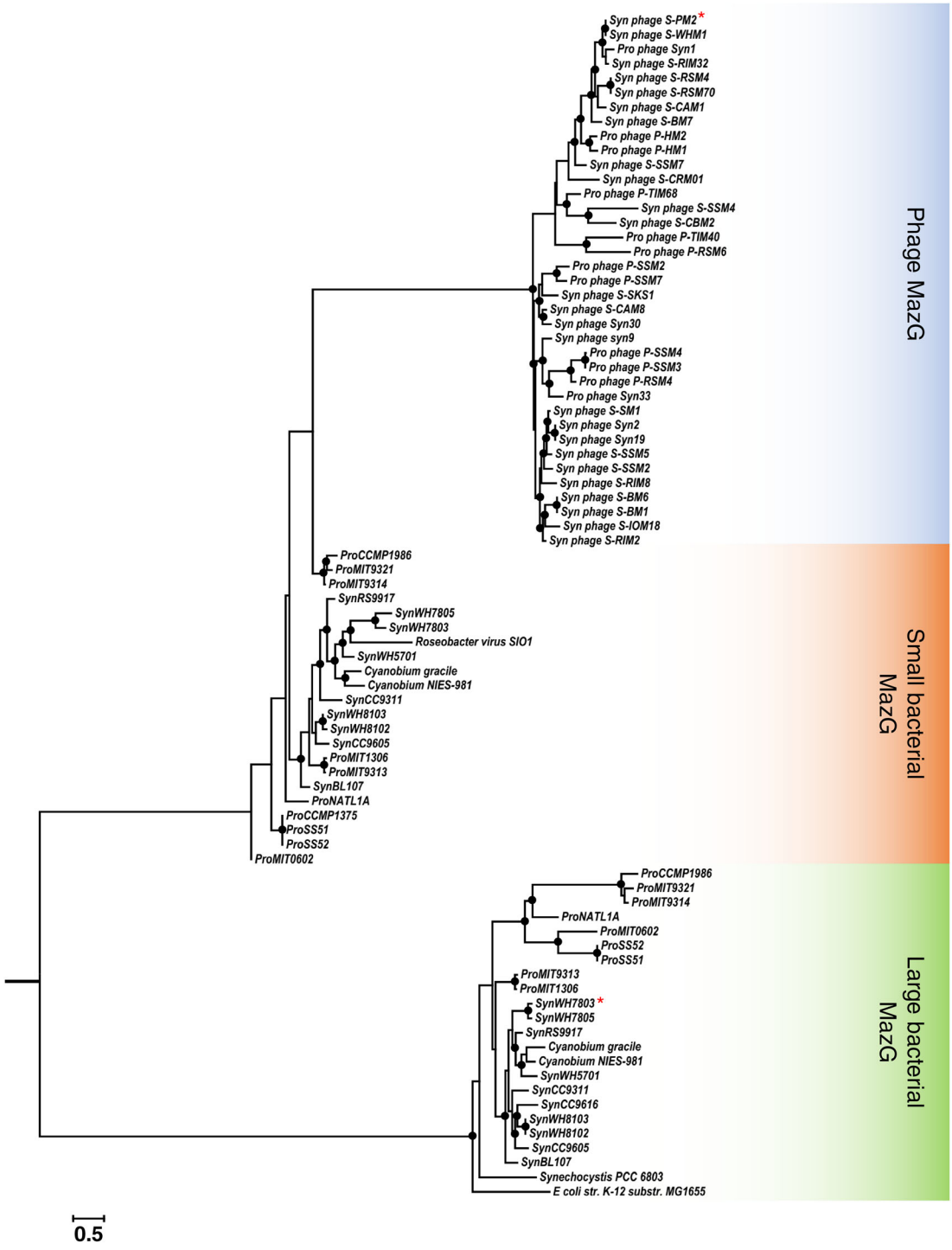
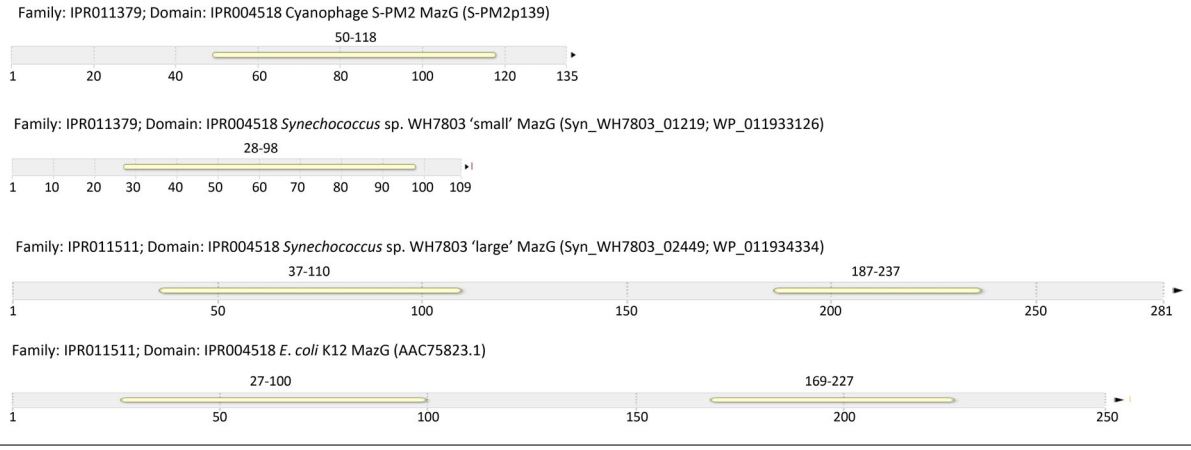


Fig. 1. Maximum likelihood phylogenetic tree comprising 44 bacterial and 38 viral MazG sequences.
The tree was generated using the LG + G4 substitution model, automatically chosen by the Iqtree script (Nguyen *et al.,* 2015), with ultrafast bootstrap (Minh *et al.,* 2013). Bootstrap values of >70% are shown as closed circles (of 1000 iterations). The scale bar represents 0.5 substitutions/amino acid position. Syn: *Synechococcus*; Pro: *Prochlorococcus.* The red asterisks indicate the *Synechococcus* and cyanophage proteins used here.

may trick the host into mimicking a nutrient replete cellular state so that host cell physiology is optimized for macromolecular synthesis and hence cyanophage replication. The MazG protein belongs to the all-nucleoside triphosphate pyrophosphohydrolase (NTP-PPase, EC 3.6.1.8) superfamily that hydrolyzes *in vitro* all canonical nucleoside

(A)



(B)

```
E. coli K12      ----------------------------MNQIDRLLTIMQRLRDPENGCPWDKEQTFA  30
Syn_WH7803_02449 -----------MAQHA---------TVPGDQDPLRYLESVVARLRDPVNGCPWDLEQTHA  40
Syn_WH7803_01219 ---MQLNDY-----------------QRDSRTTARYPDLGSNLIYPTLGLAGEAGE-VA  38
Cyanophage S-PM2 MSKVNFERYQEFVSEVTSDASTNFVDFADRIGELDREGANIERLLTAGVGINAEGGE-FL  59
                                                 .*     *   :  :

E. coli K12      TIAPYTLEETYEVLDAIARDFDDLRGELGDLLFQVVFYAQMAQEEGRFDFNDICAAISD  90
Syn_WH7803_02449 SLVPYVLEEAHEVADAIRHGDDRHLKEELGDLLLQVVLHARIGAENNRFDLDAIATTISD  100
Syn_WH7803_01219 EKVKKL---IRDRNGVVDERFKQDLALELGDVLWYVAQ----LSAELGLTLDDVGRMNLE  91
Cyanophage S-PM2 EIIKKM---VFQGK-PWNRDNREHLIIELGDIMWYVAQ----ACIALGVSFDDVISGNVK  111
                       :      .    .*  ****::  *.           . :: :     .

E. coli K12      KLERRHPH----VFADSSAENSSEVLARWEQIKTEERAQKAQH--------SALDDIPRS  138
Syn_WH7803_02449 KLIRRHPH----VFGEARAENTEAVRLSWEAIKAAERAEQSGGEQSSSPLSDQLAGKVRG  156
Syn_WH7803_01219 KLKSRSTRGTLHGEGDH------R-----------------------------------  109
Cyanophage S-PM2 KLEKRYPGGEFDVFYSENRSADDR----------------------------------  135
                 **  *          .

E. coli K12      LPALMRAQKIQKRCANVGFDWTTLGPVVDKVYEEIDEVMYEARQAVVDQAKLEEEMGDLL  198
Syn_WH7803_02449 QPALAAAMTISRKAAKAGFEWDAIDGVWGKVQEELDELKEAI--ASGDRRHAQDELGDVL  214
Syn_WH7803_01219 ------------------------------------------------------------  109
Cyanophage S-PM2 ------------------------------------------------------------  135

E. coli K12      FATVNLARHLGTKAEIALQKANEKFERRFREVERIVAARGLEMTGVDLETMEEVWQQVKR  258
Syn_WH7803_02449 FTLVNVARWCGLDPEEGLAATNQRFLDRFSRVESAL---NGDLQGRSIQELEALWQQAKA  271
Syn_WH7803_01219 ------------------------------------------------------------  109
Cyanophage S-PM2 ------------------------------------------------------------  135

E. coli K12      QEIDL-----  263
Syn_WH7803_02449 AIRAENTQSS  281
Syn_WH7803_01219 ----------  109
Cyanophage S-PM2 ----------  135
```

Fig. 2. A. InterProScan5-predicted (Jones *et al.,* 2014) pyrophosphatase catalytic domains in cyanophage S-PM2 MazG, 'small' *Synechococcus* sp. WH7803 MazG (Syn_WH7803_01219), 'large' *Synechococcus* sp. WH7803 MazG (Syn_WH7803_02449) and *E. coli* MazG orthologues. Numbers above each domain represent the position of amino acids in each of the domains.
B. ClustalW pairwise alignment of *E. coli*, 'large' *Synechococcus* sp. WH7803, 'small' *Synechococcus* sp. WH7803 and cyanophage S-PM2 MazG orthologues.

triphosphates into monophosphate derivatives and pyrophosphate (PPi) (Moroz *et al.,* 2005; Galperin *et al.,* 2006; Lu *et al.,* 2010). Here, we set out to purify the cyanophage S-PM2 MazG protein as well as a *Synechococcus* host MazG to assess their activity and ability to hydrolyse (p)ppGpp, canonical and noncanonical nucleotides.

Results

Picocyanobacterial host and cyanophage MazG proteins are phylogenetically distinct (Fig. 1) and with an origin of the cyanophage MazG outside the cyanobacteria since the closest proposed homologue to date is a *Chloroflexus* protein (Bryan *et al.,* 2008; Sullivan *et al.,* 2010). Picocyanobacteria encode two genes annotated as MazG, a 'large' MazG version similar to that found in most bacteria, and a 'small' version which is similar in size to the cyanophage gene (Fig. 2). The 'large' MazG version has two predicted catalytic regions functionally annotated as MazG family domains (IPR004518) whilst the 'small' MazG and cyanophage proteins have only one (Fig. 2). In order to assess the hydrolytic activity of the host and cyanophage MazG proteins we cloned into *E. coli*, over-expressed and purified the host *Synechococcus* sp. WH7803 MazG, using the 'large' MazG version (Syn_WH7803_02449) as a proxy for other host bacterial MazG proteins, and the cyanophage S-PM2 MazG (Fig. 3; for experimental details see Supporting Information). The activity of the cyanophage and *Synechococcus* host MazG proteins was assessed using increasing concentrations of a range of nucleotide and deoxyribonucleotide substrates using 1 μg of the purified protein, and the amount of free phosphate resulting from enzyme activity measured using the PiPER pyrophosphate assay kit (ThermoFisher Scientific; see Supporting Information). This allowed determination of K_m, V_{max} and

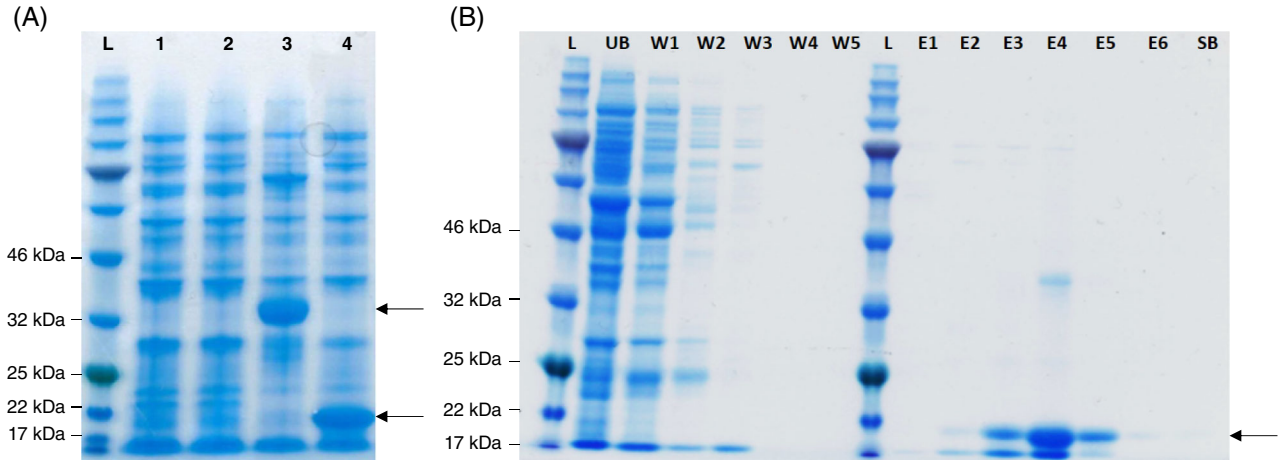


Fig. 3. A. SDS-PAGE analysis of *E. coli* whole cell lysates expressing *Synechococcus* sp. WH7803 'large' MazG (lanes 1 and 3) and cyanophage S-PM2 MazG proteins (lanes 2 and 4). L: Protein molecular weight marker ladder. Lanes 1 and 2 un-induced, lanes 3 and 4 IPTG-induced. Arrows indicate the positions of the overexpressed proteins.
B. SDS-PAGE analysis showing purification of the cyanophage S-PM2 MazG protein from *E. coli*. L: Protein molecular weight marker ladder. UB: The unbound fraction (proteins that did not bind to the column). W1–W5: fractions washed off the column with binding buffer. E1–E6: Fractions eluted with increasing concentrations of imidazole (30 mM, 50 mM, 100 mM, 150 mM, 200 mM and 300 mM respectively). SB – stripping buffer. The arrow indicates the position of the over-expressed cyanophage S-PM2 MazG protein.

K_{cat} values for each protein across a range of substrates (Table 1). K_m values of the *Synechococccus* sp. WH7803 'large' MazG and cyanophage S-PM2 MazG proteins were generally in the low mM range for a range of nucleotides and deoxyribonucleotides, similar to MazG K_m values reported from other bacteria for these substrates (Lu *et al.,* 2010). The measured V_{max} of the *Synechococcus* host MazG was highest when incubated with dTTP, whilst the viral MazG exhibited highest activity when incubated with the deoxyribonucleotides dGTP and dCTP (Fig. 4). In addition to these standard nucleotides, the viral MazG protein was also incubated with the 'aberrant' nucleotides dUTP, 2-hydroxy-dATP and 8-oxo-dGTP. dUTP is one of the most common of these mutagenic nucleotides, produced as a by-product of thymine biosynthesis (Galperin *et al.,* 2006), whilst 2-hydroxy-dATP and 8-oxo-dGTP are mutagenic nucleotides produced as a result of intracellular oxidative stress (Kamiya and Kasai, 2000; Galperin *et al.,* 2006). Interestingly, the V_{max} values of the viral MazG when incubated with dUTP, 2-hydroxy-dATP and 8-oxo-dGTP were not significantly different to those of the canonical nucleotides (Table 1; Fig. 4), whilst the Km values for these substrates were higher (Table 1), suggesting that dGTP and dCTP are the preferred substrates of the cyanophage MazG protein.

In order to directly assess whether the *Synechococcus* and cyanophage MazG proteins play a role in (p)ppGpp metabolism we performed both hydrolysis and DRaCALA binding assays (Corrigan *et al.,* 2016), using ^{32}P-labelled GTP, ppGpp and pppGpp. In both assays, neither the *Synechococcus* nor cyanophage MazG showed any

Table 1. Kinetic parameters of enzymatic activity of Synechococcus WH7803 and cyanophage S-PM2 MazG protein.

	V_{max} (nmol/μg/min)		K_m (mM)		K_{cat} (min^{-1})	
	Synechococcus sp. WH7803	Cyanophage S-PM2	*Synechococcus* sp. WH7803	Cyanophage S-PM2	*Synechococcus* sp. WH7803	Cyanophage S-PM2
dATP	1.8 (±0.28)	1.62 (±0.19)	0.3 (±0.09)	1.2 (±0.21)	126.12 (±19.35)	62.97 (±7.44)
dCTP	3.81 (±0.36)	8.86 (±0.2)	0.14 (±0.03)	1.16 (±0.04)	267.68 (±25.02)	344.68 (±7.72)
dTTP	6.57 (±0.19)	5.68 (±0.2)	ND	1.23 (±0.06)	461.04 (±13.43)	221.00 (±7.78)
dGTP	0.64 (±0.25)	10.29 (±0.25)	0.85 (±0.07)	0.14 (±0.01)	45.16 (±17.6)	400.35 (±9.91)
ATP	2.55 (±0.35)	2.28 (±0.24)	0.63 (±0.23)	1.43 (±0.36)	179.27 (±24.4)	88.7 (±9.41)
CTP	1.96 (±0.14)	2.51 (±0.17)	1.2 (±0.21)	0.85 (±0.11)	137.81 (±9.81)	97.48 (±6.68)
GTP	0.7 (±0.13)	0.3 (±0.02)	0.26 (±0.02)	ND	49.46 (±9.19)	11.67 (±0.6)
UTP	3.02 (±0.2)	3.31 (±0.18)	1.33 (±0.3)	0.6 (±0.37)	221.07 (±6)	128.75 (±7.12)
dUTP	-	4.22 (±0.34)	-	3.24 (±1.55)	-	296.42 (±23.72)
2-hydroxy d-ATP	-	1.65 (±0.06)	-	4.86 (±1.13)	-	115.65 (±3.95)
8-oxo-dGTP	-	ND	-	ND	-	ND

The values in brackets represent *SE* based on three replicates. ND – not detected; – not measured.

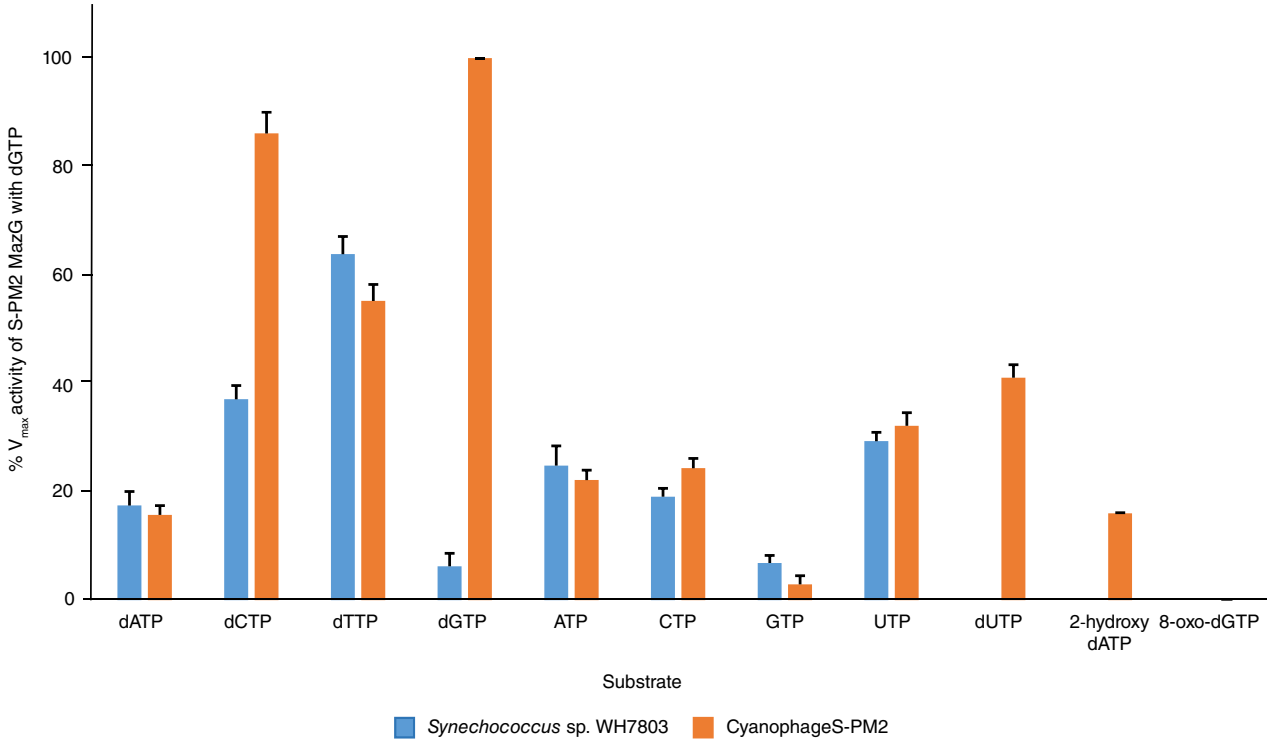


Fig. 4. Relative maximal activity (V_{max}) of the *Synechococcus* sp. WH7803 'large' MazG and cyanophage S-PM2 MazG proteins against a range of canonical and noncanonical nucleotide and deoxyribonucleotide substrates, normalized to the activity of the cyanophage S-PM2 MazG using dGTP as a substrate.
Error bars represent the standard error based on three replicate experiments.

binding or hydrolysis activity against (p)ppGpp (Fig. 5A), whilst hydrolysis activity was confirmed for both orthologues against ^{32}P-labelled GTP (Fig. 5B).

Discussion

Although, the presence and identity of AMGs in bacteriophage genomes is widely appreciated (Millard *et al.,* 2009; Sullivan *et al.,* 2010; Crummett *et al*., 2016) the specific role of many of these genes has not been resolved. Here, we sought to elucidate the activity of the cyanophage MazG protein given its hypothesized role as a more general modulator of the host stringent response, and with previous data suggesting cyanophage can modulate intracellular levels of (p)ppGpp in infected freshwater cyanobacteria (Borbély *et al.,* 1980).

Our results showed, however, that neither the *Synechococcus* nor cyanophage MazG protein demonstrated detectable hydrolytic activity towards ppGpp or pppGpp (Fig. 5), suggesting these two proteins do not actively modulate the stringent response via direct hydrolysis of magic spot nucleotides. Nevertheless, we cannot rule out a role for these proteins in regulating the stringent response indirectly through hydrolysis of other nucleotide substrates, for example GTP. Whilst the role of the 'small' *Synechococcus* host MazG also requires clarification in this respect, it is potentially the predicted bifunctional *Synechococcus* sp. WH7803 SpoT orthologue (SynWH7803_2342) that serves the role of regulating alarmone levels during the stringent response in these organisms, a protein known to both synthesize and hydrolyse (p)ppGpp in other bacteria (see, e.g. Murray and Bremer, 1996; Hogg *et al.,* 2004). Interestingly, there were distinct differences in the hydrolytic activities of the *Synechococcus* host and cyanophage S-PM2 MazG proteins towards other canonical and noncanonical nucleotides (Fig. 4 and Table 1) with much higher V_{max} values of the viral MazG towards dGTP and dCTP coupled with a much higher affinity of the viral MazG for dGTP compared to its host counterpart. Such different kinetic parameters mirror differences in %GC content between the cyanophage and *Synechococcus* host genomes, with the former possessing a GC content of 37.7% (Mann *et al.,* 2005) and the latter a GC content of 60.2% (Dufresne *et al.,* 2008). With this in mind, we suggest that the substrate specificity of the viral MazG allows it to preferentially hydrolyse dGTP and dCTP deoxyribonucleotides from the high GC content host *Synechococcus* genome allowing for their recycling and ultimately facilitating replication of the AT-rich cyanophage genome. Whether such a mechanism is applicable to, or modified in, *Prochlorococcus* infecting cyanophage

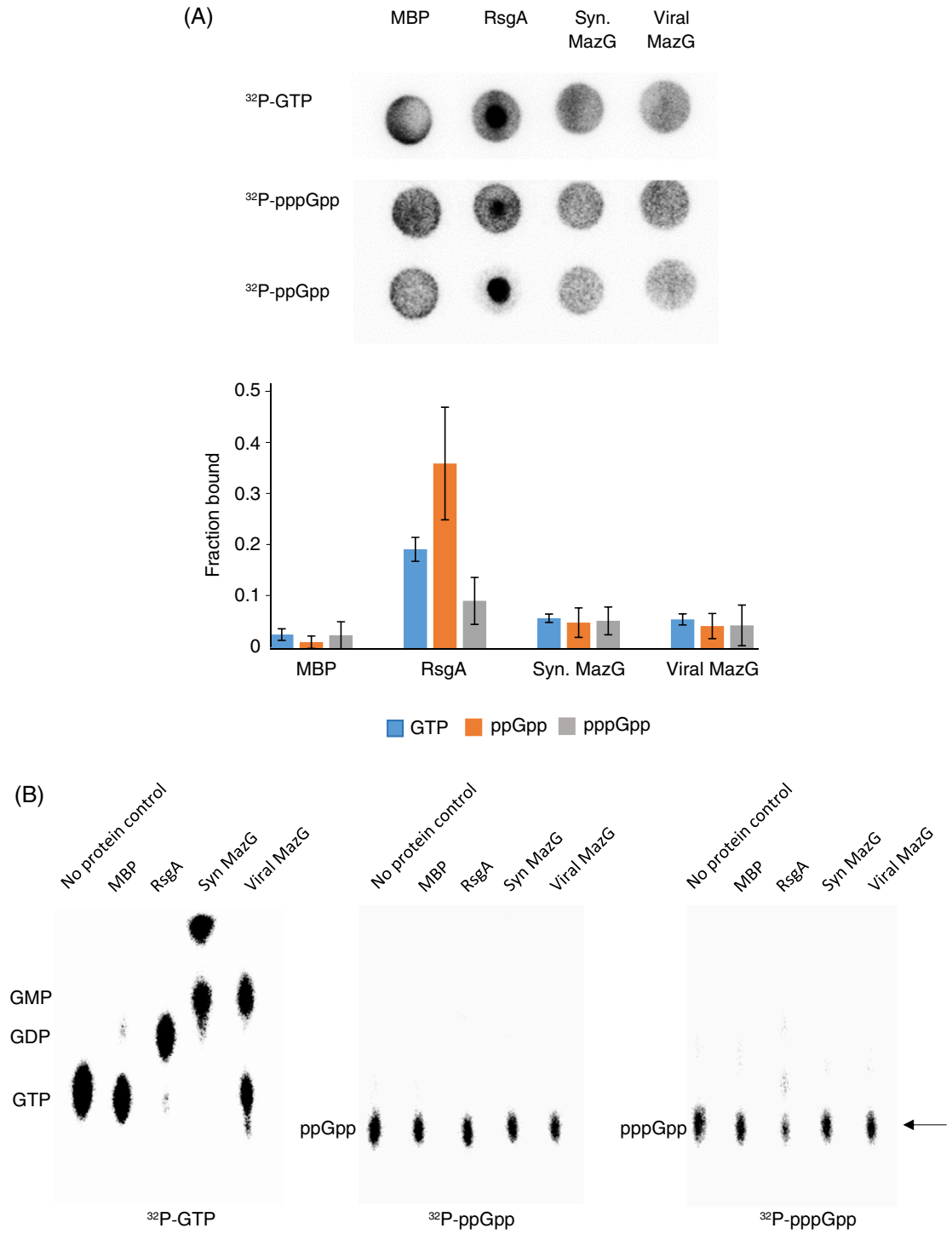


Fig. 5. A. Upper panel: DRaCALA binding assays, using ^{32}P-labelled GTP, ppGpp and pppGpp incubated with purified *Synechococcus* sp. WH7803 'large' MazG and cyanophage S-PM2 MazG proteins. MBP – maltose binding protein, used as a negative control. RsgA –purified RsgA protein from *S. aureus*, used as a positive control. Syn MazG: *Synechococcus* sp. WH7803 'large' MazG. Viral MazG: cyanophage S-PM2 MazG. Lower panel: Bar chart representation of the fraction of substrate bound to each protein, as measured by densitometry. Syn. MazG: *Synechococcus* sp. WH7803 'large' MazG. Viral MazG: cyanophage S-PM2 MazG. Error bars represent the standard deviation of three experimental replicates.
B. Hydrolysis assay using purified *Synechococcus* sp. WH7803 'large' MazG (Syn MazG), cyanophage S-PM2 MazG (Viral MazG), MBP and RsgA proteins with ^{32}P-labelled GTP, ppGpp and pppGpp. The arrow highlights the absence of hydrolysis of ^{32}P-labelled ppGpp and pppGpp substrates.

whose genomes generally possess a similar %GC content (Sullivan *et al.,* 2005; Limor-Waisberg *et al.,* 2011) remains to be determined. Certainly, it is well known that following infection with cyanophage, the host genome is rapidly degraded (Doron *et al.,* 2016). Moreover, analysis of viral metagenomes has shown an enrichment of metabolic pathways involved in pyrimidine and purine metabolism as well as in DNA replication (Enav *et al.,* 2014), emphasizing the importance of these pathways during viral infection.

Our work with the viral MazG thus highlights that cyanophage genomes appear exquisitely suited to promote degradation of the host genome in order to reuse its building blocks to replicate the viral genome.

Acknowledgements

B. R. was in receipt of a Chancellor's International PhD Scholarship from the University of Warwick. R.M.C. was supported by funding from the Wellcome Trust and Royal Society grant 104110. This work was also supported by the Natural Environment Research Council through Research Grants NE/J02273X/1 and NE/N003241/1. Bioinformatics analysis was carried out using MRC CLIMB Infrastructure (grant MR/L015080/1).

References

Baran, N., Goldin, S., Maidanik, I., and Lindell, D. (2018) Quantification of diverse virus populations in the environment using a polony method. *Nat Microbiol* **3**: 62–72.

Biller, S.J., Berube, P.M., Lindell, D., and Chisholm, S.W. (2015) *Prochlorococcus*: the structure and function of collective diversity. *Nat Rev Microbiol* **13**: 13–27.

Borbély, G., Kari, C., Gulyás, A., and Farkas, G.L. (1980) Bacteriophage infection interferes with guanosine 3′-diphosphate-5′-diphosphate accumulation induced by energy and nitrogen starvation in the cyanobacterium *Anacystis nidulans*. *J Bacteriol* **144**: 859–864.

Breitbart, M., Thompson, L.R., Suttle, C.A., and Sullivan, M. B. (2007) Exploring the vast diversity of marine viruses. *Oceanography* **20**: 135–139.

Bryan, M.J., Burroughs, N.J., Spence, E.M., Clokie, M.R.J., Mann, N.H., and Bryan, S.J. (2008) Evidence for the intense exchange of MazG in marine cyanophages by horizontal gene transfer. *PLoS One* **3**: e2048.

Clokie, M.R., and Mann, N.H. (2006) Marine cyanophages and light. *Environ Microbiol* **8**: 2074–2082.

Clokie, M.R., Millard, A.D., and Mann, N.H. (2010) T4 genes in the marine ecosystem: studies of the T4-like cyanophages and their role in marine ecology. *Virology J* **7**: 291.

Corrigan, R.M., Bellows, L.E., Wood, A., and Gründling, A. (2016) ppGpp negatively impacts ribosome assembly affecting growth and antimicrobial tolerance in Gram-positive bacteria. *Proc Natl Acad Sci USA* **113**: E1710–E1719.

Crummett, L.T., Puxty, R.J., Weihe, C., Marston, M.F., and Martiny, J.B. (2016) The genomic content and context of auxiliary metabolic genes in marine cyanomyoviruses. *Virology* **499**: 219–229.

Dammeyer, T., Bagby, S.C., Sullivan, M.B., Chisholm, S.W., and Frankenberg-Dinkel, N. (2008) Efficient phage mediated pigment biosynthesis in oceanic cyanobacteria. *Curr Biol* **18**: 442–448.

Doron, S., Fedida, A., Hernández-Prieto, M.A., Sabehi, G., Karunker, I., Stazic, D., *et al*. (2016) Transcriptome dynamics of a broad host-range cyanophage and its hosts. *ISME J* **10**: 1437–1455.

Dufresne, A., Ostrowski, M., Scanlan, D.J., Garczarek, L., Mazard, S., Palenik, B.P., *et al*. (2008) Unravelling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. *Genome Biol* **9**: R90.

Enav, H., Mandel-Gutfreund, Y., and Béjà, O. (2014) Comparative metagenomic analyses reveal viral-induced shifts of host metabolism towards nucleotide biosynthesis. *Microbiome* **2**: 9.

Fridman, S., Flores-Uribe, J., Larom, S., Alalouf, O., Liran, O., Yacoby, I., *et al*. (2017) A myovirus encoding both photosystem I and II proteins enhances cyclic electron flow in infected *Prochlorococcus* cells. *Nat Microbiol* **2**: 1350–1357.

Galperin, M.Y., Moroz, O.V., Wilson, K.S., and Murzin, A.G. (2006) House cleaning, a part of good housekeeping. *Mol Microbiol* **59**: 5–19.

Gross, M., Marianovsky, I., and Glaser, G. (2006) MazG—a regulator of programmed cell death in *Escherichia coli*. *Mol Microbiol* **59**: 590–601.

Hogg, T., Mechold, U., Malke, H., Cashel, M., and Hilgenfeld, R. (2004) Conformational antagonism between opposing active sites in a bifunctional RelA/SpoT homologue modulates (p)ppGpp metabolism during the stringent response. *Cell* **117**: 57–68.

Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., *et al*. (2014) InterProScan 5: genome-scale protein function classification. *Bioinformatics* **30**: 1236–1240.

Kamiya, H., and Kasai, H. (2000) 2-hydroxy-dATP is incorporated opposite G by *Escherichia coli* DNA polymerase III resulting in high mutagenicity. *Nucleic Acids Res* **28**: 1640–1646.

Limor-Waisberg, K., Carmi, A., Scherz, A., Pilpel, Y., and Furman, I. (2011) Specialization versus adaptation: two strategies employed by cyanophages to enhance their translation efficiencies. *Nucleic Acids Res* **39**: 6016–6028.

Lin, X., Ding, H., and Zeng, Q. (2016) Transcriptomic response during phage infection of a marine cyanobacterium under phosphorus-limited conditions. *Environ Microbiol* **18**: 450–460.

Lindell, D., Sullivan, M.B., Johnson, Z.I., Tolonen, A.C., Rohwer, F., and Chisholm, S.W. (2004) Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. *Proc Natl Acad Sci USA* **101**: 11013–11018.

Lindell, D., Jaffe, J.D., Johnson, Z.I., Church, G.M., and Chisholm, S.W. (2005) Photosynthesis genes in marine viruses yield proteins during host infection. *Nature* **438**: 86–89.

Lu, L.D., Sun, Q., Fan, X.Y., Zhong, Y., Yao, Y.F., and Zhao, G.P. (2010) Mycobacterial MazG is a novel NTP pyrophosphohydrolyase involved in oxidative stress response. *J Biol Chem* **285**: 28076–28085.

Mann, N.H., Cook, A., Millard, A., Bailey, S., and Clokie, M. (2003) Marine ecosystems: bacterial photosynthesis genes in a virus. *Nature* **424**: 741.

Mann, N.H., Clokie, M.R.J., Millard, A., Cook, A., Wilson, W. H., Wheatley, P.J., *et al*. (2005) The genome of S-PM2, a 'photosynthetic' T4-type bacteriophage that infects marine *Synechococcus* strains. *J Bacteriol* **187**: 3188–3200.

Millard, A., Clokie, M.R.J., Shub, D.A., and Mann, N.H. (2004) Genetic organization of the *psbAD* region in phages infecting marine *Synechococcus* strains. *Proc Natl Acad Sci USA* **101**: 11007–11012.

Millard, A.D., Zwirglmaier, K., Downey, M.J., Mann, N.H., and Scanlan, D.J. (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**: 2370–2387.

Minh, B.Q., Nguyen, M.A.T., and von Haeseler, A. (2013) Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* **30**: 1188–1195.

Moroz, O.V., Murzin, A.G., Makarova, K.S., Koonin, E.V., Wilson, K.S., and Galperin, M.Y. (2005) Dimeric dUTPases, HisE, and MazG belong to a new superfamily of all-α NTP pyrophosphohydrolases with potential 'housecleaning' functions. *J Mol Biol* **347**: 243–255.

Mühling, M., Fuller, N.J., Millard, A., Somerfield, P.J., Marie, D., Wilson, W.H., *et al*. (2005) Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. *Environ Microbiol* **7**: 499–508.

Murray, K.D., and Bremer, H. (1996) Control of *spoT*-dependent ppGpp synthesis and degradation in *Escherichia coli*. *J Mol Biol* **259**: 41–57.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., and Minh, B. Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* **32**: 268–274.

Puxty, R.J., Millard, A.D., Evans, D.J., and Scanlan, D.J. (2016) Viruses inhibit CO_2 fixation in the most abundant phototrophs on earth. *Curr Biol* **26**: 1585–1589.

Roitman, S., Hornung, E., Flores-Uribe, J., Sharon, I., Feussner, I., and Béjà, O. (2018) Cyanophage-encoded lipid desaturases: oceanic distribution, diversity and function. *ISME J* **12**: 343–355.

Scanlan, D.J., Ostrowski, M., Mazard, S., Dufresne, A., Garczarek, L., Hess, W.R., *et al*. (2009) Ecological genomics of marine picocyanobacteria. *Microbiol Mol Biol Rev* **73**: 249–299.

Sullivan, M.B., Waterbury, J.B., and Chisholm, S.W. (2003) Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. *Nature* **424**: 1047–1051.

Sullivan, M.B., Coleman, M.L., Weigele, P., Rohwer, F., and Chisholm, S.W. (2005) Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. *PLoS Biol* **3**: e144.

Sullivan, M.B., Huang, K.H., Ignacio-Espinoza, J.C., Berlin, A.M., Kelly, L., Weigele, P.R., *et al*. (2010) Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. *Environ Microbiol* **12**: 3035–3056.

Suttle, C.A. (2007) Marine viruses - major players in the global ecosystem. *Nat Rev Microbiol* **5**: 801–812.

Suttle, C.A., and Chan, A.M. (1994) Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Appl Environ Microbiol* **60**: 3167–3174.

Thompson, L.R., Zeng, Q., Kelly, L., Huang, K.H., Singer, A. U., Stubbe, J., and Chisholm, S.W. (2011) Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. *Proc Natl Acad Sci USA* **108**: E757–E764.

Traxler, M.F., Summers, S.M., Nguyen, H.T., Zacharia, V.M., Hightower, G.A., Smith, J.T., and Conway, T. (2008) The global, ppGpp-mediated stringent response to amino acid starvation in *Escherichia coli*. *Mol Microbiol* **68**: 1128–1148.

Zeidner, G., Bielawski, J.P., Shmoish, M., Scanlan, D.J., Sabehi, G., and Béjà, O. (2005) Potential photosynthesis gene recombination between *Prochlorococcus* and *Synechococcus* via viral intermediates. *Environ Microbiol* **7**: 1505–1513.

Zeng, Q., and Chisholm, S.W. (2012) Marine viruses exploit their host's two-component regulatory system in response to resource limitation. *Curr Biol* **22**: 124–128.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supplementary Information