Brief Report

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

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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 (Mühleng et al., 2005). Cyanophage can also influence ocean biogeochemistry by modifying host metabolism during the infection process, such as the shutdown of CO₂ 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 (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

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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...

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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 protein (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* *sp.* WH7803, ‘small’ *Synechococcus* *sp.* WH7803 and cyanophage S-PM2 MazG orthologues.
The values in brackets represent SE based on three replicates. ND – not detected; – not measured.

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binding or hydrolysis activity against (p)ppGpp (Fig. 5A), whilst hydrolysis activity was confirmed for both orthologues against 32P-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 non-canonical 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 Syn-echococcus 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 32P-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: Synchococcus 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 32P-labelled GTP, ppGpp and pppGpp. The arrow highlights the absence of hydrolysis of 32P-labelled ppGpp and pppGpp substrates.

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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.

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References


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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