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The effect of inhibition of host MreB on the infection of thermophilic phage GVE2 in high temperature environment

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In eukaryotes, the manipulation of the host actin cytoskeleton is a necessary strategy for viral pathogens to invade host cells. Increasing evidence indicates that the actin homolog MreB of bacteria plays key roles in cell shape formation, cell polarity, cell wall biosynthesis, and chromosome segregation. However, the role of bacterial MreB in the bacteriophage infection is not extensively investigated. To address this issue, in this study, the MreB of thermophilic *Geobacillus sp.* E263 from a deep-sea hydrothermal field was characterized by inhibiting the MreB polymerization and subsequently evaluating the bacteriophage GVE2 infection. The results showed that the host MreB played important roles in the bacteriophage infection at high temperature. After the host cells were treated with small molecule drug A22 or MP265, the specific inhibitors of MreB polymerization, the adsorption of GVE2 and the replication of GVE2 genome were significantly repressed. The confocal microscopy data revealed that MreB facilitated the GVE2 infection by inducing the polar distribution of virions during the phage infection. Our study contributed novel information to understand the molecular events of the host in response to bacteriophage challenge and extended our knowledge about the host-virus interaction in deep-sea vent ecosystems.

In most rod-shaped bacteria, the bacterial MreB, a homolog of actin[1], is employed in maintaining their morphology and guiding the peptidoglycan synthesis during elongation[2–5]. Gram-negative bacteria, such as *Escherichia coli* and *Caulobacter crescentus*, produce only a single form of MreB, whereas gram-positive bacteria such as *Bacillus subtilis* have multiple MreB-like proteins (Mbl and MreBH)[1,6,7]. Mutational analyses have provided accumulating evidence that MreB plays key roles in cell shape formation, cell polarity, cell wall biosynthesis, and chromosome segregation[1,6–14]. As visualized by fluorescence microscopy, MreB proteins have been reported to form spiral-like filamentous structures along the rod-shaped cells, underneath the cytoplasmic membrane[1,3,6,7,11,15–19]. Many studies have suggested that MreB helices act as a scaffold for proteins involved in cell wall biosynthesis, cell elongation and chromosome segregation[1,5,6,10,11,17,20]. However, some recent reports using high-resolution imaging indicate that either in *E. coli* or in *B. subtilis* MreB filaments do not run the length of the cell[21,22]. Instead, they are actually composed of small dynamic patches that move around the cell circumference and are driven by the cell wall synthesis machinery[4,7,21–25].

As reported, when the filament formation of MreB is blocked by a specific drug A22 [S-(3,4-dichlorobenzyl) isothiourea][26], the bacterial cells lose their rod shape and transform into spherical morphology[1,3,4,7,11,20,26–29]. Then a downstream abrogation in penicillin-binding protein (PBP) localization and peptidoglycan (PG) synthesis patterns can be observed[1,30]. The round-shaped cells finally die unless their growth conditions are changed[7]. For example, media with high concentrations of Mg^{2+} allow the normal growth of *B. subtilis* MreB mutant[17,31]. Considering the toxicity and indirectness of A22, a new drug named MP265 (4-chlorobenzyl chloride) is used as an MreB inhibitor in recent studies[32].

In eukaryotic cells, actin is crucial to many important cellular processes such as cell division, uptake of extracellular material, and intracellular transport[33,34]. Thus many pathogens, including viruses, have evolved to utilize host actin cytoskeleton during infection[33,34]. Because MreB is a prokaryotic homolog of actin, it is plausible that bacteriophages would take advantage of the host's MreB cytoskeleton during the virus infection. The main life cycle of bacteriophage contains several steps, including adsorption, DNA injection, metabolism transition, DNA replication, phage morphogenesis, package and lysis of the host[35]. It is documented that the *B. subtilis* MreB is involved in the DNA replication of bacteriophage phi 29[6,36]. However, the role of cytoskeletal protein MreB in

other steps of phage infection has not extensively investigated. In particular, the MreB protein has not been characterized in the phage infection of thermophiles.

In deep-sea hydrothermal vents, thermophiles comprise the basis of the food chain of these deep-sea ecosystems[37]. It is believed that the most significant players in deep-sea hydrothermal vents are thermophilic viruses including archaeal viruses and bacteriophages. Viruses may be the major causes of vent thermophile mortality[37–39].

To reveal the role of MreB in the infection of thermophilic bacteriophage GVE2 in its host *Geobacillus sp.* E263, a gram-positive thermophile from a deep-sea hydrothermal field[40], the polymerization of MreB protein was inhibited and then the phage infection was evaluated. The results showed that the host MreB played an essential role in the adsorption of GVE2 and the replication of its genome in a high temperature environment.

Results

Effects of MreB on GVE2 infection. To characterize the role of MreB in the GVE2 infection, the MreB gene was cloned from *Geobacillus sp*. E263. The results showed that the E263 strain contained MreB gene (Fig. 1A), the sequence of which was highly conserved with that of mesophilic bacteria (data not shown), suggesting that MreB in thermophiles shared similar/same functions as that in mosephilic bacteria. In mosephilic bacteria, it is reported that the filament formation of MreB could be inhibited by A22 or MP265[26,32]. Therefore the MreB-specific drugs A22 and MP265 were used to inhibit the polymerization of MreB in *Geobacillus sp.* E263 cells. The confocal microscopy data presented that the thermostable MreB-GFP was distributed in *Geobacillus sp.* E263 cells as helical structures, while the GFP alone showed no helix (Fig. 1B). When A22 or MP265 was presented, the MreB-GFP could not form the helical conformation (Fig. 1B). The results indicated that both of A22 and MP265 could inhibit the MreB polymerization in thermophiles as in mosephilic bacteria. As assayed, the optimal concentrations of A22 and MP265 were 40 μg/m and 70 μg/ml, respectively.

The GVE2 virions were purified and observed to show the characteristic shape of *Siphoviridae* bacteriophage (Fig. 1C). Then the E263 cells were treated with A22 or MP265 and subsequently infected with the purified GVE2 at high temperature (60°C), followed by turbidity assays and plaque assays to evaluate the effects of MreB on the GVE2 infection. The results indicated that the addition of GVE2 led to a significant decrease of E263 growth rate due to the lysis ability of bacteriophage as evidenced by lower turbidity values and more plaques in samples incubated with the phage (Fig. 1D and 1E). However, the A22-treated and GVE2-infected host cells (E263 + GVE2 + A22) grew normally, showing that the inhibition of MreB polymerization by A22 resulted in inefficient GVE2 infection. When the A22 in the A22-treated bacteria was removed and the bacteria were simultaneously infected with GVE2 [E263 + GVE2 + A22 (removed)], the turbidity values and plaques of the bacteria significantly decreased, suggesting that MreB was no longer inactive and that phage infection could proceed (Fig. 1D and 1E). The MP265 yielded the same results as A22 (Fig. 1D and 1E). The data presented that MreB played an essential role in the bacteriophage infection at high temperature and that this role could be withdrawn by addition of A22 or MP265.

Roles of MreB in adsorption and replication of GVE2. In an attempt to reveal the roles of MreB in GVE2 infection, the adsorption of GVE2 and replication of GVE2 genome were evaluated. In the GVE2 adsorption analysis, the MOI of GVE2 virions was 0.01. The results indicated that the copies of the free phages (phages not absorbed on E263 cell surfaces) were significantly increased in the A22-treated and GVE2-infected E263 cells (E263 + GVE2 + A22) at 15 min onwards post-infection of GVE2 (Fig. 2A). The results showed that MreB played an important role in the absorption of GVE2 during virus infection. When A22 in the A22-treated bacteria was removed [E263 + GVE2 + A22 (removed)], the number of copies of free phages in this treatment was not significantly different from that in the positive control GVE2 only (Fig. 2A). The data supported the conclusion that MreB was required for the adsorption of GVE2 in the host cells.

It was found that when the MreB polymerization was inhibited by A22, the GVE2 genome copy numbers were significantly lower than those in samples with GVE2 only (Fig. 2B), indicating the importance of MreB in the GVE2 genome replication. The results revealed that the recovery of MreB polymerization by removal of A22 led to significant increases of GVE2 genome copies (Fig. 2B). One-step growth curve assays showed that the number of plaques was significantly decreased for the treatment E263 + GVE2 + A22 compared with those of the treatments E263 + GVE2 and E263 + GVE2 + A22 (removed) (Fig. 2C), indicating that MreB might play essential roles in the GVE2 genome replication, particle assembly and host cell lysis. As assayed, MP265 yielded the similar results as A22 (Fig. 2A, B and C).

Effects of MreB on the localization of GVE2 virions in host cells during GVE2 infection. In order to characterize the effects of MreB on the distribution of GVE2 virions in host cells during phage infection, the A22-treated or MP265-treated E263 cells were infected with GVE2, followed by examination with confocal microscopy. The results showed that when the MreB polymerization was inhibited by A22 or MP265, the rod-shaped bacterial cells were changed into spherical cells (Fig. 3A). However, the removal of the drug led to the recovery of bacterial cell shape (Fig. 3A). The data indicated the essential role of the MreB polymerization in cell morphological change of bacteria. It was revealed that the morphological change of host cells led to the change of the GVE2 distribution in host cells during phage infection (Fig. 3B). GVE2 virions preferred to localize in the cell poles of its host at the early stage of infection. However, the virions were distributed around the host cells when the MreB polymerization was inhibited by A22 or MP265 (Fig. 3B). As controls, the samples were labeled with anti-GST antibody, and no signal was observed (data not shown). These results indicated that the cytoskeleton protein MreB, required for the morphology of bacteria, affected the distribution of phage in host cells during phage infection.

Confocal microscopy data revealed that the percentage of GVE2-infected host cells was significantly decreased in the treatment E263 + GVE2 + A22 compared with that of the treatment E263 + GVE2 (Fig. 3C). The findings suggested that the polymerized MreB could facilitate the GVE2 infection by affecting the distribution of virions during the phage infection.

To evaluate the colocalization of MreB and GVE2, the thermostable MreB-GFP fusion protein was overexpressed in GVE2-infected E263 cells. The results revealed that the MreB-GFP was colocalized with GVE2 during the phage infection, while the controls presented no colocalization of GFP and GVE2 (Fig. 3D), suggesting that the polymerization of MreB benefited the infection of GVE2. To determine the direct involvement of MreB in the polar localization of the GVE2 virions, the MreB-GFP -overexpressed E263 cells were treated with A22 or MP265, followed by infection with GVE2. The confocal microscopy results revealed that MreB-GFP was colocalized with the GVE2 virions and was distributed around the cells in the presence of A22 or MP265 (Fig. 3E). In this case, the GVE2 virions were not localized in the cell poles of its host (Fig. 3E), suggesting that MreB was directly involved in the polar localization of the virions. Collectively, these findings indicated that MreB played an essential role in the phage infection by direct interaction with the phage.

Discussion

Most studies on the interaction between virus and cell cytoskeleton protein come from eukaryotes. Viruses have evolved several ways to

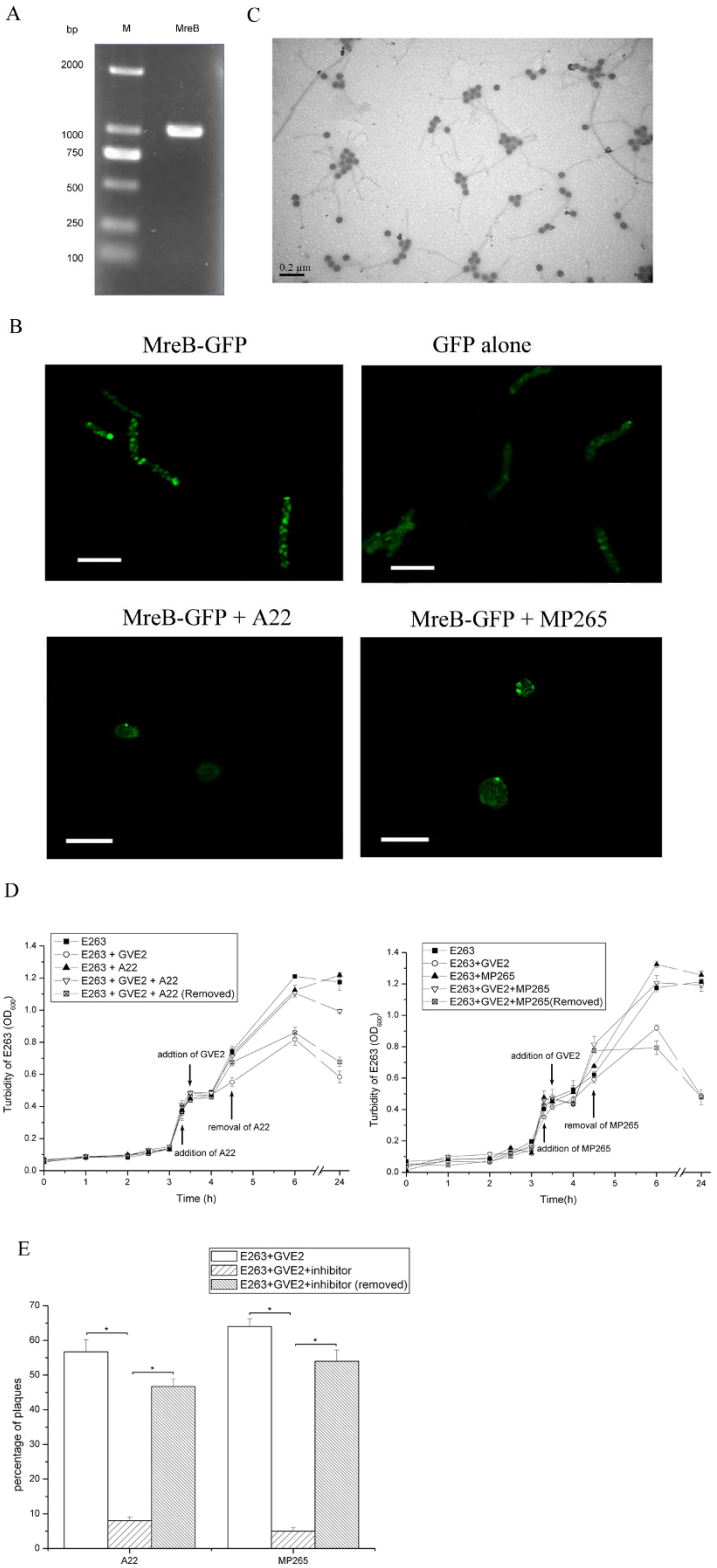

Figure 1 | Effects of MreB on GVE2 infection. (A) Cloning of MreB gene from thermophile *Geobacillus sp.* E263. M, DNA marker. (B) Effects of A22 and MP65 on the MreB polymerization in vivo. MreB-GFP was overexpressed in E263 cells. Then the cells were treated with A22 or MP265, followed by examination with confocal microscopy. The E263 cells overexpressing GFP only (GFP alone) were used as controls. Scale bar, 4 μm. (C) Examination of purified GVE2 virions by transmission electron microscope (TEM). Scale bar, 0.2 μm. (D) Turbidity assay of GVE2 infection. Turbidity assay was conducted to evaluate the interaction between MreB and GVE2 during the phage infection. The treatments were indicated at the top. At different time after the culture of E263, the optical densities (OD_{600}) of all treatments were measured by spectrophotometer. Data were shown as mean ± standard deviation (SD) of triplicate assays and were representatives of three independent experiments. (E) Plaque assay of GVE2 infection. Plaque assay was performed to evaluate the GVE2 particle assembly and host cell lysis. The bacteria were treated with GVE2 and A22 or MP265, followed by examination of percentage of plaques. Data were representatives of three independent experiments. The statistically significant differences between treatments were indicated with asterisks (*$P < 0.05$).

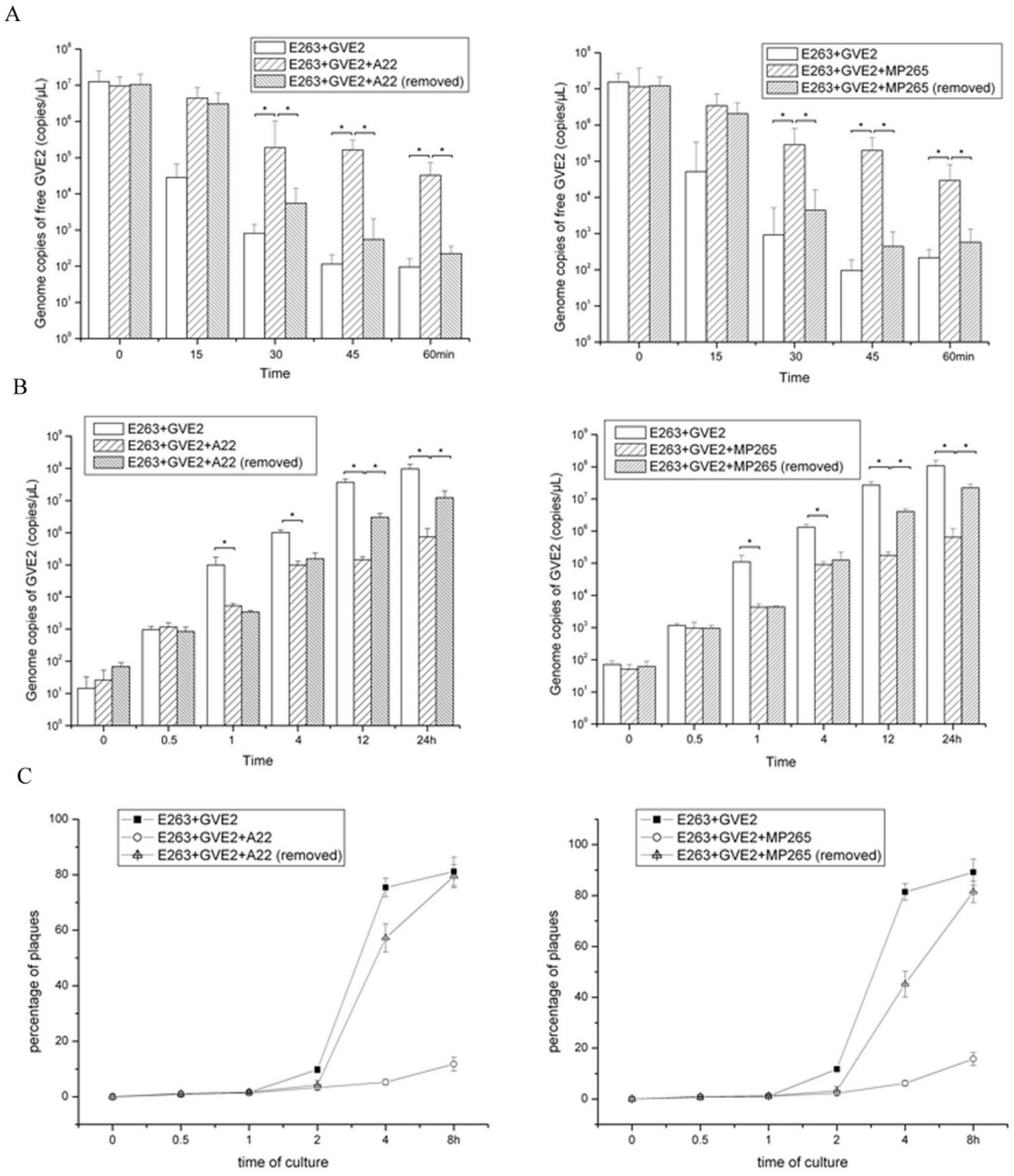

Figure 2 | Roles of MreB in GVE2 adsorption and replication. (A) Effects of MreB on the GVE2 absorption. In the presence of Ca^{2+} and Mn^{2+} ions, E263 cells were mixed with A22 or MP265 to inhibit the MreB polymerization, followed by GVE2 infection at MOI of 0.01. For the treatment E263 + GVE2 + A22 (removed) or E263 + GVE2 + MP265 (removed), A22 or MP265 was removed at 30 min after addition of A22 or MP265. Then the cells were infected with GVE2. At different times post-infection, the bacteria were collected. After centrifugation, the supernatant was subjected to quantitative real-time PCR to detect free phages. Data were representatives of three independent experiments. (B) Roles of MreB in the GVE2 replication. E263 cells were infected with GVE2, followed by treatment with A22 or MP265. For the treatment E263 + GVE2 + A22 (removed) or E263 + GVE2 + MP265 (removed), A22 or MP265 was removed at 30 min after addition of A22 or MP265. The treated bacteria were collected at different times post-infection and lysed at 99°C. After centrifugation, the supernatant was subjected to quantitative real-time PCR for the detection of GVE2 genome copies. Data were shown as mean ± SD of triplicate assays. The statistically significant differences between treatments were indicated with asterisks (*$P < 0.05$). (C) One-step growth curve of GVE2. E263 cells were incubated with A22 or MP265 and then infected with GVE2. At different time after culture, the bacteria were subjected to plaque assays, followed by examination of plaques. Data were shown as mean ± SD of triplicate assays and were representatives of three independent experiments.

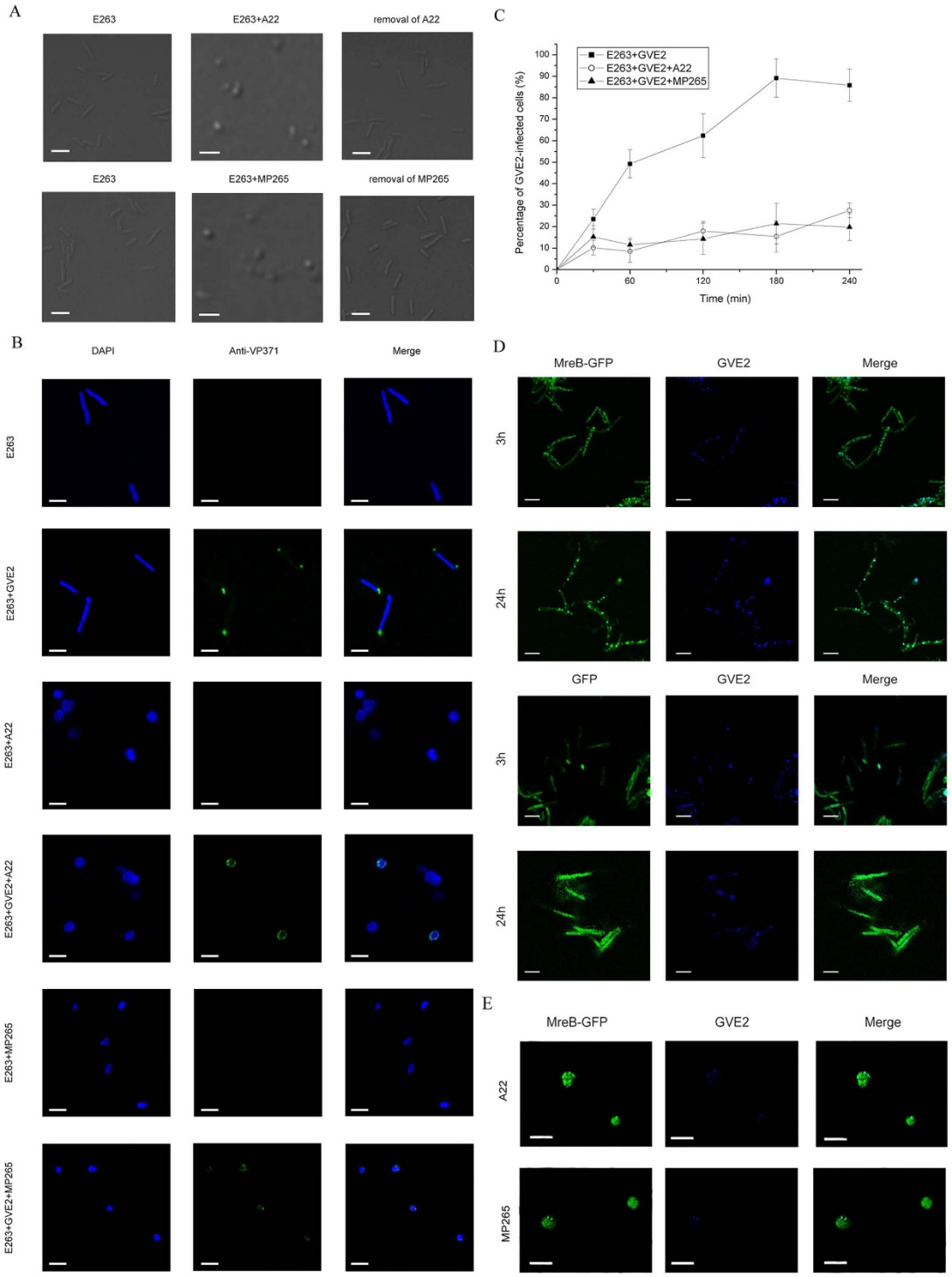

Figure 3 | Effects of MreB on the distribution of GVE2 virions in host cells during phage infection. (A) The effect of MreB inhibitor (A22 or MP265) on cell morphology. The drug A22 or MP265 was mixed with cultures of E263 at the mid log phase. At 2 min after drug inoculation, the bacteria were collected and examined with phase contrast microscopy. Then A22 or MP265 was removed and the bacterial shapes were examined at 2 min after removal of the drug. Scale bar, 4 μm. (B) Localization of GVE2 in host cells. The E263 cells were treated with A22 or MP265 to inhibit the MreB polymerization, followed by infection of GVE2. At 1 h post-infection, the E263 cells were treated with the anti-VP371 antibody to label GVE2 virions and with DAPI to label the host genome, respectively. Then the cells were examined with confocal microscopy. Nucleotides appeared as blue and virions appeared as green. Scale bar, 4 μm. (C) Effects of MreB on the GVE2 infection. The A22-treated or MP265-treated and GVE2-infected E263 cells were labeled with the anti-VP371 antibody and DAPI. At different times post-infection, the percentage of GVE2-infected cells were evaluated based on fluorescence images. Each point was shown as mean ± SD of triplicate assays. (D) Colocalization of GVE2 and MreB in E263 cells. The MreB-GFP was overexpressed in E263 cells, followed by infection of GVE2. The GFP only was used as a control. At different time post-infection, the cells were examined with confocal microscopy. GVE2 virions appeared as blue and MreB-GFP appeared as green. The numbers indicated the time post-infection with GVE2. Scale bar, 4 μm. (E) The involvement of MreB in the polar localization of the GVE2 virions. The MreB-GFP-overexpressed E263 cells were treated with A22 or MP265, followed by infection with GVE2. At 24 h post-infection, the cells were examined with confocal microscopy. GVE2 virions appeared as blue and MreB-GFP appeared as green. Scale bar, 4 μm.

exploit the host actin cytoskeleton by using its essential functions in eukaryotic cells, in particular, the uptake and short-range intracellular transport[33,34].

At present, homologues of at least three eukaryotic cytoskeletal protein families (actin, tubulin, and intermediate filaments) have been characterized in bacteria[6,15]. Phages have co-evolved with their host bacteria, thus, it's not surprising that bacteriophages can take advantage of their host's MreB cytoskeletal systems, which are known scaffolds for some key processes of bacterial cells[6]. It is believed that viruses exploit host actin to invade their hosts in eukaryotes[6,41,42]. In bacteria, however, the roles of cytoskeletal protein MreB in phage-bacterium interactions have not been extensively investigated. Our study showed that during the GVE2 infection the host MreB was required for the adsorption of phage and the replication of phage genome in the host cells.

Adsorption of bacteriophage, the first step of phage infection process, involves the recognition and contact of phage with the host cell surface. In general, adsorption of phage occurs in two steps, a reversible step and a subsequent irreversible binding step[43]. The first contact of phage with its host cell surface is usually reversible binding. So the reversible step allows for the dissociation of phage from its host. Subsequently, the phage attaches irreversibly to specific receptors on the host cell surface, leading to the injection of the phage genome from its capsid into the host cell. Although DNA injection and irreversible adsorption has been characterized, it is still contentious[43,44]. Phage adsorption to its host cell surface needs a specific interaction between the phage tail proteins and host receptors[45]. Receptors are exposed in the outer membrane of Gram-negative bacteria and in the thick peptidoglycan cell wall of Gram-positive bacteria[45]. Phage adsorption to Gram-positive bacteria is still poorly understood. It has been reported that the teichoic acid in the bacterial cell wall is essential for reversible binding of several phages in *Bacillus subtilis*, such as phi 29, phi 25, and SPP1[43].

Our study revealed that the host bacterial MreB was involved in the replication of GVE2 genome in host cells in high temperature environment. The cytoskeleton protein MreB might function as a primary organizer of the phage DNA replication because phage DNA and replication machinery components distributed in helical structures in a MreB-dependent way[6]. As well known, the gene konck-out strategy is conventionally employed to investigate the function of a gene. However, the molecular biology of thermophiles is not intensively investigated, resulting in the lack of gene knock-out system, gene overexpression system and protein labeling platforms at high temperature. To reveal the role of MreB in the phage infection, the gene knock-out strategy was conducted, but it was not successfully carried out in this study, suggesting that the deficit of the MreB might be lethal for the thermophiles. These issues merit to be further investigated. In this context, the requirement of host MreB in the phage infection process resulted from the involvement of MreB in the adsorption and replication of GVE2 in its host cells.

During the infection process, bacteriophages bind preferentially to host cell poles in some Gram-negative and Gram-positive bacteria[44,46]. For example, phage lambda needs a polar localized inner membrane protein ManY to help the injection of phage genomic DNA. Therefore, it usually shows a polar adsorption at low multiplicities of infection[44,46]. In the case of phage SPP1, both reversible adsorption step and irreversible binding to YueB occur preferentially near the cell poles, which are related to the initiation of DNA replication[44,47]. It is evident that the MreB protein is involved in the pole targeting of phage in bacterial cells[48]. In our study, the confocal microscopic images revealed that MreB played an important role in the polar distribution of phage in host cells. The MreB polymerization was required to keep the morphology of bacterial cells and the polymerized MreB facilitated the phage infection.

This study indicated that MreB was an essential factor in both adsorption of GVE2 and replication of GVE2 genome. However, the mechanism of those processes and the detailed role of MreB in GVE2 infection are still unknown. The molecular events in bacteriophage infection process in high temperature environment need to be characterized in future. Thermophiles, the basis of the food chain in deep-sea hydrothermal vent, power the vent biological communities with the source of energy. In the vent ecosystem, the most significant players in nutrient and energy cycling are thermophilic viruses[36,49,50]. Our findings provided insights into the interaction between bacteriophage and host thermophile in high temperature environment. This would be helpful to reveal roles of bacteriophages in controlling bacteria population and carbon turnover in deep-sea hydrothermal vent ecosystems.

Methods

Infection of *Geobacillus sp.* E263 by GVE2 and purification of GVE2 virions. The deep-sea thermophile *Geobacillus sp.* E263 and its thermophilic bacteriophage GVE2 were isolated in our previous studies[37,40]. The host E263 was cultured at 60°C in TTM medium (0.2% NaCl, 0.4% yeast extract, 0.8% tryptone; pH 7.0) supplemented with 25 mM $MgSO_4$[17,31]. The host strain cultures in the mid log phase (OD_{600} = 0.4) were infected with GVE2 at a multiplicity of infection (MOI) of 0.01, 0.5 or 5.

The GVE2 virions were purified as described previously[37]. Virus samples were examined under a transmission electron microscope (JEOL 100 CXII) for purity.

Dosages of the MreB inhibitors. To determine the dosages of the MreB inhibitor, A22 [S-(3,4-dichlorobenzyl) isothiourea, Merck, Germany] or MP265 (4-chlorobenzyl chloride, Alfa Aesar, USA) with different concentrations (0.1–100 μg/ml) were mixed with cultures of E263 at the mid log phase (OD_{600} = 0.4). At different time after A22 or MP265 inoculation, the bacteria were collected and examined by microscopy to evaluate the optimal dosages.

Turbidity assay. To assess the infection of E263 cells by GVE2, a turbidity assay was performed as previously described[37]. E263 cells were cultured at 60°C. When the OD_{600} of the cultured bacteria was 0.3, the bacteria were mixed with A22 (final concentration 40 μg/ml) or MP265 (final concentration 70 μg/ml). Ten minutes later, the bacteria were infected with the purified GVE2 virions at a MOI of 5. To evaluate the effects of the removal of A22 or MP265 in the A22-treated bacteria or the MP265-treated bacteria on the GVE2 infection, the bacteria of the treatment E263 + GVE2 + A22 (removed) or E263 + GVE2 + MP265 (removed) were cultured for 1 h after the GVE2 infection and then centrifuged at 18,000 × g for 5 min to remove A22 or MP265. Subsequently the pelleted bacteria were resuspended and cultured in fresh TTM medium. At different times after the culture of E263, the optical densities (OD_{600}) of all treatments were measured by spectrophotometer (UV-1700 Shimadzu Corporation, Japan). The experiments were repeated three times.

Plaque assay. The E263 cultures (OD_{600} = 0.4) were mixed with A22 (40 μg/ml) or MP265 (70 μg/ml) and then incubated for 30 min. Then the bacteria were mixed with GVE2 virions at an MOI of 0.5 and subsequent with TTM soft agar medium (TTM medium with 0.5% agar). The soft media were spread on TTM solid medium plate, followed by incubation at 60°C overnight. For the treatment E263 + GVE2 + A22 or E263 + GVE2 + MP265, A22 or MP265 was dissolved in the TTM soft agar medium. For the treatment E263 + GVE2 + A22 (removed) or E263 + GVE2 + MP265 (removed), there was no A22 or MP265 in the TTM soft agar medium. Finally the plaques were examined.

GVE2 adsorption assay. E263 cultures at OD_{600} of 0.4 were supplemented with 10 mM $CaCl_2$ and 15 mM $MnCl_2$ at 60°C. At the same time, 40 μg/ml of A22 or 70 μg/ml of MP265 was added into the cultures. After culture of E263 for 30 min, the bacterial cells were collected and infected with purified GVE2 virions at an MOI of 0.01. For the treatment E263 + GVE2 + A22 (removed), A22 was removed 30 min after addition of A22 (as described above). The treatment E263 + GVE2 + MP265 (removed) was carried out accordingly. Then the cells were infected with GVE2. At different time postinfection, the bacteria were collected. After 5 s of vortex, the bacteria were incubated for 5 min at room temperature, and then centrifuged at 15,000 × g for 5 min. The supernatant was subjected to quantitative real-time PCR to detect the free (non-adsorbed) phages.

GVE2 replication analysis. Cells of E263 at OD_{600} of 0.3 were infected with purified GVE2 virions at a MOI of 5. After culture at 60°C for 30 min, the cells were mixed with 40 μg/ml of A22 or 70 μg/ml of MP265. For the treatment E263 + GVE2 + A22 (removed) or E263 + GVE2 + MP265 (removed), A22 or MP265 was removed at 30 min after addition of A22 or MP265 as described above. The treated bacteria were collected at different times postinfection, vortexed for 5 s, lysed for 20 min at 99°C, and centrifuged for 10 s. The supernatant was subjected to quantitative real-time PCR for the detection of GVE2 genome copies. The experiments were conducted three times.

One-step growth curve of GVE2. The E263 cells were suspended in 800 μl of fresh TTM medium and then incubated with GVE2 at an MOI of 0.5 for 30 min at 4°C.

Subsequently 10 μl of the antibody against VP371 (a capsid protein of GVE2) was added to terminate the adsorption. The mixture was centrifuged at 12,000 × g for 10 min and the pellets containing the GVE2-infected cells were resuspended in 20 ml of TTM medium. The bacteria were mixed with A22 (40 μg/ml) or MP265 (70 μg/ml) and incubated for 10 min at room temperature. For the treatment E263 + GVE2 + A22 (removed) or E263 + GVE2 + MP265 (removed), the bacteria were washed with TTM media by centrifugation at 1,000 × g to remove A22 or MP265. All the above bacteria were cultured at 60°C. At different time after culture, the bacteria were subjected to plaque assays. The experiments were biologically repeated three times.

Quantitative real-time PCR. Real-time PCR was conducted to quantify the GVE2 genome copies in cell lysate using GVE2-specific primers (5′-ATCGGTTGTA-CTAACTTAAC-3′ and 5′-GCTTG TCGTATTCCTTATC-3′) and GVE2-specific TaqMan fluorogenic probe (5′-FAM CC GTCTTGTTCGTTGTCTCTGC-Eclipse-3′). The genomic DNA of GVE2 was collected from the GVE2-infected *Geobacillus sp.* E263 samples by heating at 99°C for 20 min. The real-time PCR was conducted as described previously[51].

Protein recombinant expression in *E. coli*, antibody preparation and ntibody labeling. Protein recombinant expression, antibody preparation and antibody labeling were carried out as before[37]. Briefly the vp371 gene of GVE2[52] was cloned into pGEX-4T-2 vector (Novagen, Germany) and expressed in *E. coli* BL21 (DE3) as a glutathione S-transferase (GST)-tagged fusion protein. The recombinant VP371 protein was used as an antigen to immunize mice. The immunoglobulin G (IgG) fraction of the antiserum was purified with protein A-Sepharose (Bio-Rad, USA) and stored at −80°C until use. As determined by enzyme-linked immunosorbent assay, the antiserum titer was 1 : 10,000. The specificity of antibody was confirmed using Western blotting with the recombinant protein. Then the antibody labeling was conducted to label the antibody using fluorescent dyes[37].

Phase contrast microscopy. E263 cells at exponential growth phase were treated with 40 μg/ml of A22 or 70 μg/ml of MP265. At 30 min after the addition of the drug, E263 cells were harvested by centrifugation at 1000 × g for 1 min. Subsequently the A22-treated, MP265-treated and drug-free E263 cells were examined with a phase contrast microscopy (Nikon, Japan).

Immunofluorescence microscopy. Overnight cultures of E263 were diluted with TTM medium containing 0.01 M $MgCl_2$ at 1 : 100 and then grown at 60°C. When the OD_{600} of bacteria reached 0.3, the bacteria were treated with 40 μg/ml of A22 or 70 μg/ml of MP265. After culture for 30 min, the cells were infected with GVE2 at an MOI of 5. At different times post-infection, the GVE2-infected bacteria were collected. For imaging, the E263 cells were immobilized with methanol for 30 min at 4°C. Then the labeled anti-VP371 or anti-GST antibody was added to the immobilized cells that were permeabilized by acetone (30 min, 4°C), and the cells were incubated overnight at 4°C. The cells were labeled with 10 μg/ml of 4′, 6-diamidino-2-phenylindole (DAPI) (Invitrogen) for 10 min. Subsequently 10 μl of samples were dripped on slides (Sigma, USA) covered with a thin 1% agarose film and examined under a Leica TCS SP5 confocal microscope (Germany). The digital images were acquired by and analyzed using LAS AF version 2.0.0 software.

Effects of A22 and MP265 on the MreB polymerization. In order to elucidate the effects of A22 and MP265 on the MreB polymerization, a thermosensitive *sgsE* promoter[51], a thermostable GFP gene (Los Alamos National Laboratory, USA) and the MreB gene of E263 were cloned into the pNW33N vector (Bacillus Genetic Stock Center, USA) to make the pNW33N-sgsE_p-MreB-GFP construct. Then the E263 cells were transformed with the plasmid and cultured at 65°C. E263 cells at exponential growth phase were treated with 40 μg/ml of A22 or 70 μg/ml of MP265 for 30 min. After immobilization with methaol for 30 min, the drug-treated and drug-free E263 cells were examined with a Leica TCS SP5 confocal microscope (Germany).

Colocalization of MreB and GVE2. The E263 cells were transformed with the pNW33N-sgsE_p-MreB-GFP plasmid and cultured at 65°C. Overnight culture of E263 harboring MreB-GFP was diluted with TTM medium at 100 folds, and continued to grow at 65°C. When the OD_{600} reached 0.6, the bacteria were infected with purified GVE2 at an MOI of 5. At different times, the bacteria were collected and the GVE2 virions were labeled with the anti-VP371 antibody as described above. Subsequently the bacteria were examined under a Leica TCS SP5 confocal microscope (Germany).

Statistical analysis. The numerical data from three independent experiments were analyzed by one-way analysis of variance (ANOVA) to calculate the mean and standard deviation (SD) of the triplicate assays.

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Author contributions

M.J., Y.C. and C.X. performed the experiments under the supervision of X.Z., M.J. and Y.C. wrote the manuscript. All authors reviewed the manuscript.

Additional information

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