In Vivo Assembly of Phage φ29 Replication Protein p1 into Membrane-associated Multimeric Structures

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The mechanisms underlying compartmentalization of prokaryotic DNA replication are largely unknown. In the case of the Bacillus subtilis phage φ29, the viral protein p1 enhances the rate of in vivo viral DNA replication. Previous work showed that p1 generates highly ordered structures in vitro. We now show that protein p1, like integral membrane proteins, has an amphiphilic nature. Furthermore, immunoelectron microscopy studies reveal that p1 has a peripheral subcellular location. By combining in vivo chemical cross-linking and cell fractionation techniques, we also demonstrate that p1 assembles in infected cells into multimeric structures that are associated with the bacterial membrane. These structures exist both during viral DNA replication and when φ29 DNA synthesis is blocked due to the lack of viral replisome components. In addition, protein p1 encoded by plasmid generates membrane-associated multimers and supports DNA replication of a p1-lacking mutant phage, suggesting that the pre-assembled structures are functional. We propose that a phage structure assembled on the cell membrane provides a specific site for φ29 DNA replication.

In both eukaryotes and prokaryotes, the use of large organizing structures to bring together replication factors seems to be a general mechanism to enhance the efficiency of the replication process. In eukaryotes, chromosomal DNA replication occurs at numerous locations within the nucleus. Each site constitutes a replication factory containing many polymerizing machines working on different templates (1). Replication factories fixed to a nucleoskeleton have been visualized by electron microscopy techniques (2). This finding supports a replication model in which DNA polymerases are immobilized by attachment to larger structures, and DNA is pulled through (stationary replisome model) (3). Nuclear substructures also appear to have a central role in replication of DNA viruses, like the nuclear matrix in adenovirus DNA replication (4–6). Attachment of replication complexes to specific structures has also been reported for eukaryotic positive-strand RNA viruses. In this case, replication occurs in close association with intracellular membranes of diverse origin (e.g. endoplasmic reticulum, lysosome, chloroplast) (7). Furthermore, the discovery that chromosomal DNA replication in Bacillus subtilis and Escherichia coli takes place at a centrally located, stationary replication factory has led to the proposal that the bacterial replisome might be anchored to an underlying structure, presumably the bacterial membrane (8–10). However, despite these observations, little is known on the mechanisms that position the replication complexes at specific structures.

In the case of the B. subtilis phage φ29, cell fractionation studies showed that parental viral DNA-membrane complexes are formed near the onset of viral DNA replication. Moreover, formation of these complexes required the synthesis of early viral-encoded proteins (11). Thus, viral proteins appear to be involved in the attachment of φ29 DNA to the bacterial membrane. The genome of φ29 is a linear double-stranded DNA molecule with a terminal protein (TP) (12) covalently linked at each 5’-end. Its replication starts at either DNA end, where the replication origins are located, by a protein-primeing mechanism. In vitro, this initiation mechanism requires the formation of a heterodimer between a free molecule of TP, which acts as a primer, and the φ29 DNA polymerase (12). The viral protein p1 (85 residues) enhances the rate of in vivo φ29 DNA replication, playing a critical role when bacteria are growing at high, rather than low, temperatures (13). Quantitative immunoblotting revealed that p1 is present in about 10^4 molecules per cell at early stages of infection, and it increases up to 10^5 molecules per cell at late stages (14). Protein p1 has interesting features such as the following. (i) It is recovered in membrane fractions of infected cells (14). (ii) It assembles into highly ordered structures in vitro (13), and (iii) its N-terminal region binds to primer TP in vitro (15). Although p1 has no sequence homology to cytoskeletal elements, we found that a truncated p1 protein that lacks the N-terminal 33 amino acids assembles in vitro into large polymers that show a parallel array of longitudinal protofilaments (13). These structures, examined by negative-stain electron microscopy, resemble polymers formed under particular in vitro conditions by FtsZ, which forms a ring-like structure that mediates bacterial cell division (16, 17). However, unlike FtsZ, p1 polymerization is not regulated by GTP hydrolysis. The ability of protein p1 to generate highly ordered structures in vitro, and its presence in membrane fractions of infected cells, led us to propose that p1 could assemble in vivo to form a multimeric structure in close association with the bacterial membrane. Verification of this hypothesis is essential to understand the role of p1 in φ29 DNA replication.

In this work, we show that p1, like integral membrane pro-
teins (18), has an amphiphilic nature. Moreover, immunoelectron microscopy studies reveal that protein p1 is located at or close to the bacterial membrane. By combining in vivo chemical cross-linking and cell fractionation techniques, we also show that during 29 DNA replication there are large homomeric p1 complexes associated with the bacterial membrane. Furthermore, protein p1 encoded by plasmid generates membrane-associated multimers and supports DNA replication of a p1-tanking mutant phage, suggesting that the pre-assembled structure is functional. We discuss the possible role of the p1 structures in the formation of 29 DNA replication compartments.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Bacteriophages, and Plasmids—B. subtilis 110NA, a non-suppressor (su) strain, and B. subtilis MO-99, a suppressor strain (suA), were used (19). The 29 sus4 (56), sus3 (91) and sus2 (513) mutant phages were isolated by Moreno et al. (19). The 29 sus1 (629) mutant phage was isolated by Reilly et al. (20). To construct plasmid pPR55.5, a HinCl-HinClII DNA fragment from 29 (coordinates 1447–850) (21), which contains the p1-encoding gene, was inserted into the T4 DNA polymerase-treated Sphl site of the B. subtilis expression vector pPR55 (22). Plasmid-containing cells were grown in LB medium (23) supplemented with 100 µg/ml ampicillin and 0.8% glucose. Phase partitioning of hydrophilic from amphiphilic proteins (18) was carried out at 30 °C, enabling protein solubilization with 2% (v/v) Triton X-114 in phosphate-buffered saline containing 0.1 M NaCl. Synthesis of Viral DNA—The method used to measure synthesis of viral DNA under one step phase growth conditions was as described (22). Basically, total intracellular DNA was isolated at different times after infection, and analyzed by 0.8% agarose gel electrophoresis. Gels were stained with ethidium bromide. Images were obtained with the UVITEC UV/WL 23 system. DNA band with respect to that of proteinase K-treated reaction.

Phage Growth under One Step Conditions — Cultures were exponentially grown to ~10^7 cells/ml, and then infected at a multiplicity of infection of 5–10. After 10 min of incubation with gentle shaking, unadsorbed phages were eliminated by centrifugation of the infected culture. Cells were resuspended in the same volume of medium and incubated with vigorous shaking for the indicated time.

Synthesis of Viral DNA—The method used to measure synthesis of viral DNA under one step phase growth conditions was as described (22). Basically, total intracellular DNA was isolated at different times after infection, and analyzed by 0.8% agarose gel electrophoresis. Gels were stained with ethidium bromide. Images were obtained with the UVITEC UV/WL 23 system. Bands corresponding to unit length DNA were quantified using the Image quant v.1.1 program. The relative amount of viral DNA refers to the intensity of the intracellular 29 DNA band with respect to that of proteinase K-treated 29 DNA (50 ng) used as internal marker.

In Vivo Chemical Cross-linking—Bacteria were washed with 50 mM Hepes, pH 8.0, and concentrated 4-fold in buffer P (50 mM Hepes, 10 mM EDTA, 0.2% sucrose, pH 8.0). The cross-linker bis(sulfosuccinimidyl) suberate (BS3) (Pierce) was dissolved in 50 mM Hepes, pH 8.0, just before use and added to the concentrated culture at different final concentrations. After incubation at room temperature for 20 min, Tris-HCl, pH 7.5, was added at a final concentration of 150 mM to quench the reaction.

Membrane Preparations—Membrane vesicles of B. subtilis cells were prepared as described (14). This method is basically described on the procedure described by Konings et al. (24).

Triton X-114 Phase Partitioning—Triton X-114 is a non-ionic detergent with a low cloud point (23 °C) enabling protein solubilization with phase partitioning of hydrophilic from amphiphilic proteins (18). Cells were concentrated 4-fold in 50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl, pH 8.0. Lysozyme was added at a final concentration of 120 µg/ml. After 10 min at room temperature, cells were sedimented by centrifugation. Detergent extraction of proteins was carried out overnight at 4 °C using 2% (w/v) Triton X-114 in phosphate-buffered saline (PBS) as described (25). Detergent-insoluble material was solubilized by centrifugation at 4 °C prior to phase separation. For phase partitioning, the sample was incubated at 37 °C for 10 min followed by centrifugation for 10 min at room temperature. The resulting aqueous and detergent phases were supplemented with Triton X-114 or PBS, respectively, and washed three times by repeating the phase separation step. Prior to SDS-PAGE (26), proteins from the phases were precipitated with 10 volumes of acetone at –20 °C for 1 h.

Western Blots—Proteins were transferred electrophoretically to Immobilon-P membranes (Millipore) using either a Trans Blot apparatus (Bio-Rad) at 100 mA and 4 °C for 90 min, or a Mini Trans Blot (Bio-Rad) at 100 mA and 4 °C for 60 min. Transfer buffer contained 25 mM Tris, 192 mM glycine, and 20% methanol. Membranes were blocked with 5% non-fat milk, 0.005% Tween 20 and 0.007% sodium azide in PBS, pH 7.2, for 30 min and 50% glycerol, in 0.1 M sodium phosphate, pH 7.2, for 30 min. Then, cells were pelleted and immediately layered onto a sterile cellulose ester membrane filter (Whatman). Wedge-shaped portions of this filter were cryofixed by plunging into liquid propane in a KF80 cryofixation unit (Reichert Jung). Freeze-substitution was performed with potassium permanganate (0.5% uranyl acetate in an automatic freeze-substitution unit (AFS, Leica) at –85 °C for 54 h. The temperature was gradually increased (5 °C/h) to –35 °C and held constant for 24 h. Samples were washed with pure methanol three times for 1 h each at –35 °C and then were infiltrated in a graded series of Lowicryl K4M/methanol mixtures (1:2 for 1 h, 1:1 for 2 h, 3:1 for 2 h). Cells were embedded in Lowicryl K4 M at –35 °C for 24 h and polymerized for 24 h under ultraviolet irradiation. During the polymerization step, the temperature was gradually increased to 22 °C. Infiltration, embedding, and polymerization steps were carried out in an automatic freeze-substitution equipment (AFS, Leica). Thin sections (70 nm) were obtained by cutting the specimen on a Reichert Jung ultracut E ultramicrotome and placed on collodion carbon-coated grids for immunogold labeling. Thin sections on the grids were blocked with TBS (30 mM Tris-HCl, pH 8.2, 150 mM NaCl) containing 5% bovine serum albumin (BSA) for 5 min. Then, they were incubated with affinity-purified p1 antibodies (diluted 1:10 in 0.1% acetylated BSA in TBS) for 4 h. Sections were incubated with 10 nm colloidal gold-labeled protein A (Biocoll) (diluted 1:25 in 1% BSA in TBS) for 45 min. The grids were washed in TBS and double-distilled water and air-dried. Finally, sections were stained with 2% aqueous uranyl acetate for 7 min and 0.2% lead citrate for 25 s. Electron micrographs were taken in a Jeol 1010 electron microscope at 80 kV.

RESULTS

Protein p1 Has an Amphiphilic Nature—To perform experiments during in vivo viral DNA replication, B. subtilis non-suppressor (suA) cells were infected with the 29 sus4 (56) mutant phage. As shown in Fig. 1A, protein p1 and 29 sus4 (56) DNA accumulated throughout the course of infection. At 30 min of infection, the intracellular level of p1 was similar to that previously measured in B. subtilis cells infected with the wild-type phage (14). In 29 sus4 (56) infected cells, the 29 regulatory protein p4 is not synthesized and, consequently, late viral transcription is not activated. The late genes encode components of the viral capsids, proteins involved in phage morphology and those required for cell lysis (27). Hence, 29 sus4 (56) DNA is replicated but it cannot be packaged into capsids.

The C-terminal sequence of p1 spanning residues Tyr^68 to Ala^84 is highly hydrophilic (Fig. 1B). Cell fractionation studies, performed with 29 sus4 (56) infected cells, showed that p1 is recovered in membrane fractions during viral DNA replication (14). Unlike the wild-type p1, a truncated p1 protein that lacks the C-terminal 43 amino acids neither associates with membranes in vivo nor self-interacts in vitro, although it interacts with the primer TP in vitro (15). We have now investigated the nature of the association between protein p1 and membranes by phase partitioning with Triton X-114, a non-ionic detergent widely used for the isolation of integral membrane proteins. This technique accurately predicts membrane localization of proteins (18, 25). To this end, at 30 min of 29 sus4 (56) infection, when viral DNA replication was taking place (Fig. 1A), whole cells were solubilized with Triton X-114, and the soluble material was subjected to phase separation. An immunoblot analysis of the aqueous and detergent phases using polyclonal antibodies against the viral proteins p1 and p6 is shown in Fig. 2. Protein p1 was found exclusively in the deter-
Peripheral Location of p1 in Infected Cells by Immunoelectron Microscopy—In a complementary approach to localize p1 in infected cells, we used immunoelectron microscopy. To increase the signal of p1, cells were fixed and processed for immunolabeling at 45 min of infection (Fig. 3A). This percentage indicates that protein p1 is located at or near the cell membrane, rather than being randomly dispersed, and are consistent with its copurification with membranes on cell fractionation (14).

Protein p1 Forms Membrane-associated Multimeric Structures during in Vivo Viral DNA Replication—Protein p1 was shown to be able to interact into highly ordered structures in vitro (13). The COILS prediction program (29) revealed that the region of p1 spanning residues Glu38 to Asn65 has a high probability (0.98) of forming a α-helical coiled-coil structure (Fig. 1B). Such a sequence functions to assemble an N-terminal truncated p1 protein (p1N33) into two-dimensional sheets (30). To investigate whether p1 assembles in vivo into multimers that associate with the bacterial membrane, we carried out in vivo chemical cross-linking experiments followed by cell fractionation. At 30 min of infection (see Fig. 1A), cells were incubated with different concentrations of BS3. This homobifunctional cross-linker reacts significantly with the ε-amino of lysine residues, which are frequent in p1 (12 residues of 85). Following this treatment, membrane fractions were obtained by a method that yields almost exclusively "right-side-out" membrane vesicles (24). Total proteins from the membrane preparations were separated by denaturing polyacrylamide gel electrophoresis and analyzed by immunoblotting using anti-p1 serum (Fig. 4). As control, membrane preparations from non-infected cells were analyzed. The anti-p1 serum cross-reacted with a host membrane-associated protein that migrated at ~50 kDa (protein X). This protein provided an internal control of specificity in the cross-linking reaction. In infected cells and in the absence of BS3, a product migrating as the p1 monomer (8.5 kDa) was detected. At low concentrations of BS3 (0.25–0.5 mM), two products migrating at 18 kDa and 30 kDa (protein X) were detected (referred to as II and III in Fig. 4). These products are likely cross-linked p1 dimers and trimers, respectively, taking into account the MW of the BS3 cross-linker (572.43 Da). At 2 mM BS3, cross-linked p1-containing material that migrates between 43 kDa and 203 kDa was also detected. According to the regular pattern, they are presumably p1 homo-complexes. At 5 mM BS3, the p1 monomeric form was not detected, and the amount of p1 complexes smaller than 70 kDa drastically decreased. This decrease correlated with an increase in the amount of higher molecular weight complexes, which were specific of infected cells. Thus, we conclude that protein p1 assembles in vivo into large membrane-associated multimers. These multimeric structures are present during viral DNA replication.

Assembly of p1 into Membrane-associated Multimers Also Occurs in the Absence of Viral Components—The φ29 replisome...
is constituted at least by a free molecule of the TP (primer protein) and the $\phi 29$ DNA polymerase. In vitro studies showed that this heterodimer interacts with the ends of the genome, where the replication origins are located. Then, replication starts by a protein-priming mechanism in which the $\phi 29$ DNA polymerase catalyzes the linkage of dAMP to the primer TP (12). We have analyzed whether membrane-associated p1 multimers are formed when initiation of in vivo $\phi 29$ DNA synthesis is blocked. To this end, B. subtilis su$^+$ cells were infected with the $\phi 29$ sus2 (513) mutant phage. Under these conditions,
initiation of phage DNA replication is blocked due to the lack of the \(\phi 29\) DNA polymerase. After 30 min of infection, cells were treated with BS\(^3\) and then fractionated. Immunoblot analysis of the membrane fractions using anti-p1 serum showed a pattern of cross-linked p1 complexes (not shown) similar to that observed during viral DNA replication (Fig. 4). The same result was obtained when \(B.\ subtilis\) sus cells were infected with the \(\phi 29\) sus3 (91) mutant phage (not shown). In this case, \(\phi 29\) DNA synthesis is blocked due to the lack of the primer TP. Thus, membrane-associated p1 multimers are also assembled in the absence of viral replisome components.

We next studied whether other viral components are required for the formation of membrane-associated p1 multimers. To this end, the p1-encoding gene was inserted into the constitutive expression vector pPR55 (plasmid pPR55.p1). The amount of p1 was -2-fold higher in cells carrying the recombinant plasmid than in 45 min \(\phi 29\) sus4 (56) infected cells, as determined by immunoblotting (not shown). When pPR55.p1-carrying cells were fractionated, p1 was recovered in the membrane fraction (Fig. 5A). Moreover, after extraction with Triton X-114, p1 was exclusively found in the detergent phase (Fig. 5B). We also examined whether p1 formed multimeric structures by treatment of pPR55.p1-carrying cells with BS\(^3\). Cells harboring pPR55 were used as control. Fig. 5C shows an immunoblot analysis of the membrane fractions using anti-p1 serum. In pPR55.p1-carrying cells, the pattern of cross-linked p1 complexes was indistinguishable from that observed in \(\phi 29\) sus4 (56) infected cells (Fig. 4). Immunoelectron microscopy studies using affinity-purified p1 antibodies confirmed the peripheral location of p1 in pPR55.p1-carrying cells (Fig. 6). Again, cells harboring pPR55 were used as control. In both cases, 1800 cross-sections on different grids were examined. In vector-carrying cells, only 8.5% of the sections were labeled, and 164 gold particles were counted (Fig. 6A). In pPR55.p1-carrying cells, the percentage of labeled sections increased 4.8-fold. Moreover, the total number of gold particles increased 9.7-fold. Most of the labeled sections (83.3%) had one, two, or three gold particles, although sections with 4 to 8 gold particles were also found. Fig. 6B shows the distribution of the gold particles on the cross-sections. In vector-carrying cells, out of the 164 particles counted, 69 were at the periphery, whereas in pPR55.p1-carrying cells, out of the 1596 particles counted, 1252 were at the periphery. Thus, the ratio between peripheral and cytoplasmic gold particles was 5-fold higher in pPR55.p1-containing cells. Cross-sections of pPR55.p1-carrying cells with peripheral gold particles are shown in Fig. 6C.

**Trans-complementation Mediated by Protein p1**—Taken together, the above results indicated that protein p1 encoded by plasmid is able to assemble into membrane-associated multimers. If these p1 structures were similar to those assembled in infected cells, we reasoned that they should support replication of a p1-lacking mutant phage. To assess this prediction, we analyzed replication of the \(\phi 29\) sus1 (629) mutant phage in different genetic backgrounds: plasmid-free suppressor (\(su^+\)) cells and non-suppressor (\(su^-\)) cells carrying either pPR55 or pPR55.p1 (Fig. 7). When pPR55-carrying \(su^-\) cells were infected with the \(\phi 29\) sus1 (629) mutant phage, no synthesis of viral DNA was detected. In contrast, phage DNA replication took place in pPR55.p1-carrying \(su^-\) cells. In these cells, the kinetics of accumulation of viral DNA was even faster than that obtained in \(su^+\) cells infected with the sus1 (629) mutant, and the amount of viral DNA accumulated at 35 min was -2-fold higher. Hence, these results suggest that the membrane-associated p1 structures formed in pPR55.p1-carrying cells are functional in trans.

**DISCUSSION**

**Assembly of p1 into Membrane-associated Multimeric Structures**—A remarkable finding of this study is that protein p1 of phage \(\phi 29\) assembles in vivo into large multimeric structures. Furthermore, these structures interact with the bacterial membrane, as supported by the following results. (i) Protein p1 has an amphiphilic nature, as determined by Triton X-114 phase partitioning. (ii) p1 copurifies with membranes on cell fractionation (14), and (iii) immunoelectron microscopy studies show that p1 has a peripheral subcellular location.

In infected cells, membrane-associated p1 structures exist both when viral DNA replication takes place and when initiation of \(\phi 29\) DNA synthesis is blocked due to the lack of viral replisome components. Membrane-associated p1 structures are...
Membrane-associated p1 Structures

also generated in cells harboring a p1-encoding plasmid, indicating that their formation does not require other viral components. The ability of p1 to self-associate has also been demonstrated in vitro. For example, protein p1 functions as a polymerization domain when it is fused to the maltose-binding protein, leading to the formation of long filamentous structures (13). In addition, a truncated p1 protein that lacks the N-terminal 33 amino acids self-interacts into two-dimensional prolate filaments. This assembly is mediated by a short α-helical-coil-coil sequence (30). The coiled-coil motif is also the main structural element in eukaryotic laminas, a class of nuclear intermediate filament proteins (31, 32). In vivo, different lamin proteins form the nuclear lamina, a thin fibrous structure immediately underlying the inner nuclear membrane of most eukaryotic cell nuclei (33). It remains to be determined what kind of structures p1 would form in vivo.

Role of the Membrane-associated p1 Structures in ϕ29 DNA Replication—Parental ϕ29 DNA-membrane complexes have been isolated from infected cells. Formation of these complexes, which were detected near the onset of viral DNA replication, required the synthesis of early ϕ29-encoded proteins (11). Our present results support an in vitro replication model in which a p1 structure assembled on the bacterial membrane provides a specific site for ϕ29 DNA replication (15). According to this model, the ϕ29 DNA replication machinery, constituted at least by the primer TP-ϕ29 DNA polymerase heterodimer, would be targeted to the membrane-associated p1 structure. This association could be achieved by protein-protein interactions between protein p1 and primer TP, since the N-terminal 42 amino acid residues of p1 are sufficient for binding to the primer TP in vitro (15). Once ϕ29 DNA replication starts by a protein-priming mechanism, the primer TP remains covalently bound to the nucleoid-associated protein p6 (14).

The role proposed for the membrane-associated p1 structure resembles the function of the eukaryotic nuclear matrix in adenovirus DNA replication. Like phage ϕ29, the genome of adenovirus is a linear double-stranded DNA with a TP covalently attached to each 5′-end. Therefore, its replication proceeds via a protein-priming mechanism (34). Cell fractionation studies indicated that adenovirus DNA is tightly bound to the nuclear matrix throughout the course of infection (35, 36). It has been shown that this association is mediated by the TP covalently bound to the DNA (37). Furthermore, the precursor of the TP, which interacts with the viral DNA polymerase and primes DNA replication, binds to the nuclear matrix both in vivo and in vitro (4). Hence, the nuclear matrix is thought to provide the structural framework on which the replication factors and DNA can bind and interact.

Comparison with other Viral Systems—Replication of eukaryotic viral genomes does not occur randomly in the infected cell but is localized to specific sites. Replication of most DNA viruses occurs within the cell nucleus. In this case, nuclear substructures seem to be involved in the formation of DNA replication compartments (5, 6, 38). For example, as mentioned above, adenovirus replicates at distinct subnuclear sites on the nuclear matrix (39, 40). In the case of positive-strand RNA viruses, the docking to specific intracellular membranes seems to be essential for the assembly of active replication complexes. Membranes appear to function not just as a way of compartmentalizing virus RNA replication, but also appear to have a central role in the organization and function of the replication machinery (7). Some progress has been made in understanding how the viral RNA replication apparatus is fixed to specific types of membranes. In Semliki Forest virus, binding of the viral replicase to endosomal/lysosomal membranes is likely mediated by the RNA-capping protein Np1, which has affinity for negatively charged phospholipids (41, 42). Moreover, the tobacco etch potyvirus 6-kDa protein is thought to be necessary for targeting viral RNA replication complexes to endoplasmic reticulum-derived membranes. This protein associates with membranes as an integral protein via a 19 amino acid hydrophobic domain (43). In contrast to those viruses, a host-encoded protein seems to act as a membrane anchor of the tobravirus replication complexes. This protein associates with membranes and interacts with the helicase domain of virus-encoded replication proteins (44). In poliovirus, electron microscopy revealed that purified RNA-dependent RNA polymerase forms planar and tubular oligomeric arrays. In addition, membraneous vesicles isolated from infected cells contain structures consistent with the presence of two-dimensional polymerase arrays on their surfaces during infection (45).

Therefore, as it happens in eukaryotic viral genomes, replication of the B. subtilis ϕ29 occurs at specific subcellular localizations. We suggest that the p1 structure assembled on the cell membrane provides an anchoring site for the ϕ29 replication complexes.

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