P335 lactococcal phages infect the Gram + bacterium Lactococcus lactis using a large multiprotein complex located at the distal part of the tail and termed baseplate (BP). The BP harbors the receptor-binding proteins (RBPs), which allow the specific recognition of saccharidic receptors localized on the host cell surface. We report here the electron microscopic structure of the phage TP901-1 wild-type BP as well as those of two mutants bppL− and bppU−, lacking BppL (the RBPs) or both peripheral BP components (BppL and BppU), respectively. We also achieved an electron microscopic reconstruction of a partial BP complex, formed by BppU and BppL. This complex exhibits a tripod shape and is composed of nine BppLs and three BppUs. These structures, combined with light-scattering measurements, led us to propose that the TP901-1 BP harbors six tripods at its periphery, located around the central tube formed by ORF46 (Dit) hexamers, at its proximal end, and a ORF47 (Tal) trimer at its distal extremity. A total of 54 BppLs (18 RBPs) are thus available to mediate host anchoring with a large apparent avidity. TP901-1 BP exhibits an infection-ready conformation and differs strikingly from the lactococcal phage p2 BP, bearing only 6 RBPs, and which needs a conformational change to reach its activated state. The comparison of several Siphoviridae structures uncovers a close organization of their central BP core whereas striking differences occur at the periphery, leading to diverse mechanisms of host recognition.

The first steps of phage infection require interactions between the phage receptor-binding proteins (RBPs)1 (1, 2) and the receptors at the host cell surface. Although some RBPs are located at the tip of fibers (3), others belong to an elongated structure, the tail spike (4, 5). In bacteriophages infecting the Gram + bacterium Lactococcus lactis such as p2 (936 group), TP901-1, and Tuc2009 (P335 group), RBPs are part of a large organelle (1–2 MDa) termed the baseplate (BP). We previously solved RBPs structures of phages p2 (6, 7) and TP901-1 (8) as well as the RBP C-terminal domain (“head domain”) of phage bIL170 (936 group) (9). It appeared that the RBP of phage TP901-1 (termed BppL, lower baseplate protein) was cleaved during crystallization, and the polypeptidic chain in the crystal structure starts either at residue 16 or at residue 32 (10). Proteolytic cleavage was also observed for the homologous RBP from Tuc2009 (11) as well as in the structure of a chimeric RBP comprising the N-terminal and linker domains of phage TP901-1 RBP fused to the C-terminal domain of phage p2 RBP (12). We demonstrated that individually expressed Tuc2009 BppU (upper baseplate protein) and BppL did not interact when mixed, and we attributed this to the proteolytic cleavage of the BppL N terminus that should normally plug into BppU (13). In contrast, co-expression of BppU and BppL yielded a well defined 3:9 complex (13).

The BP architecture of the P335 phages TP901-1 and Tuc2009 has been investigated thoroughly using mutagenesis and immunological labeling in combination with electron microscopy (EM) (14–16). These experiments allowed us to propose both identity and topology of BP components. In the context of a lactococcal phage proteome analysis, we overexpressed several p2, Tuc2009, and TP901-1 structural proteins and proposed topological models for both TP901-1 and Tuc2009 BPs based on interaction studies (11, 13). Overexpression of a p2 four-gene block, after starting the tape measure protein and ending with the RBP, made it possible to purify its complete BP. Determination of its x-ray structure revealed a Ca2+-induced conformational change that allowed receptor binding and DNA translocation through the BP (17). In this contribution, we report EM reconstructions of the TP901-1 BP from wild-type virions as well as from phage mu-
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tants bppL− or bppB−, lacking BppL or BppU plus BppL, respectively. Combining these results with the EM reconstruction of the “tripod” formed by the TP901-1 BppU-BppL complex, we assigned the positions of four different BP components (BppU, BppL, Dit, and Tal) in the EM maps. Six tripods were fitted into the wild-type BP EM map at its periphery, highlighting its hexagonal symmetry and providing 54 binding sites available for anchoring the phage to its host through phosphosugar receptors (18). This high number of binding sites results in a very large avidity effect, as illustrated by SPR (BIACore) measurements on a model system consisting of tripod/DARPins (designed ankyrin repeat protein) interactions. This might explain why such phages do not rely on a two-step binding mechanism.

EXPERIMENTAL PROCEDURES

Cloning and Protein Production—The bppL and bppB (NP_112712.1) genes as well as the whole DNA region containing the bppL and bppB genes with the intervening sequence were amplified from phage genomic DNA and cloned using the Gateway technology (Invitrogen) as described (13, 19). Plasmids were transformed in Rosetta(DE3)pLysS (Novagen) or T7 Express Iq pLysS (New England Biolabs). Cells were grown at 37 °C in Terrific Broth or M9 minimal medium until the OD reached 0.6, and then expression was induced with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside overnight at 25 °C or 17 °C. Protein purification was performed as described previously (13). Briefly, we performed two steps on an ÄKTA FPLC system, a Ni2+-nitrilotriacetic acid column (HisTrap Ni 5 ml; GE Healthcare) with a step gradient of 250 mM imidazole, followed by a preparative Superdex 200 HR 26/60 gel filtration run in 10 mM HEPES, pH7.5, 150 mM NaCl. For the ORFs 46/48/49 complex, we used a thioredoxin fusion construct implying additional purification steps (desalting, tobacco etch virus protease cleavage and Ni2+-nitrilotriacetic acid column) as reported (13).

TP901-1 BppL Crystallization and Structure Determination—Crystals of full-length BppL-TP901-1 were obtained by vapor diffusion at 293 K using the sitting- nanodrop method (20). 300 nl of protein at 5 mg/ml was mixed with 100 nl of 20% PEG 8000, 0.2 M magnesium acetate tetrahydrate, 0.1 M sodium cacodylate, pH 6.5 using a Cartesian Pixsys robot. Crystals were cryo-cooled in the mother liquor supplemented with 12.5% glycerol (v/v). They belong to space group H32 (R32 hexagonal axes) with unit cell parameters a = b = 41.71 Å, c = 465.24 Å, α = β = 90.0°, γ = 120.0°. 400 images (0.3° oscillation) were collected from a single crystal using an ADSC Quantum 4 detector on ID14-EH2 beamline (European Synchrotron Radiation Facility, Grenoble, France). Data were indexed and integrated using MOSFLM and scaled with SCALA (21). The asymmetric unit contains 1 molecule with a Matthews coefficient (V_M) of 2.05 Å³/Da (corresponding to 40% solvent) (22). Molecular replacement was performed with MOLREP (23) using the truncated BppL (residues 17–163, Protein Data Bank (PDB) code 2FOC) as search model. Refinement was performed using REFMAC (24). Data collection and refinement statistics are summarized in supplementary Table S1.

EM of the BppU-BppL Complex—Three microliters of sample at a final concentration of 0.05 mg/ml was deposited on glow-discharged carbon-coated copper grid. After 2 min in contact with carbon film, excess solution was blotted, and 4 μl of 1% uranyl acetate was added on the grid for 1 min. The grid was then dried and kept in a desiccator cabinet until observation.

Electron micrographs were recorded under low dose conditions with a JEOL 2200FS 200-kV microscope. Images were recorded at a magnification of ×50,000 with a defocus range of 0.4–1.0 μm. Micrographs were digitized on a Nikon Coolscan 9000 ED with a step size of 10 μm. The digitized images were coarsened by a factor of 2, resulting in a pixel size corresponding to 4 Å at the specimen level. Image processing was performed using the IMAGIC V software (25). Single-molecule images (18,841) were extracted semiautomatically from raw micrographs using Boxer (26) and corrected for the phase contrast-transfer function (CTF) by phase flipping. Some preferential views were selected by visual inspection and chosen as references using the multireference alignment module of the IMAGIC V software (25). Images were then grouped into classes and averaged using the multistatistical alignment procedure. The best-averaged class images were used as new references for a subsequent alignment cycle. At this stage, a C1 startup procedure was carried out to compute a first three-dimensional model. Despite iterative refinement cycles, heterogeneity was visible and revealed by comparison of class averages and projections of the three-dimensional model. This corresponded to arms opening movement in particles.

To go further in the analysis of such flexibility, the initial three-dimensional EM map was projected only for a few orientations. These projections were used as references for alignment of particle images. Images aligned for each reference were extracted and grouped together followed by a multistatistical alignment analysis. Therefore, five classes were computed and averaged. These class averages clearly revealed heterogeneity. More in details, flexibility is observed in the packing state of particles. Thus, two extreme states called packed and open conformations can be easily distinguished. Class averages corresponding to the open conformation are better resolved than those of the packed one. Consequently, we gathered all of the images related to class averages of the former conformation (8,542) before performing a classical single-particle analysis. After various iterative cycles of image alignment, classification, and reconstruction, a final three-dimensional reconstruction was obtained at 24.8 Å resolution, as estimated using the Fourier shell correlation criterion with a cutting level of 0.5 (27). The final density map includes 8,314 particles extracted from eight micrographs.

Native and Mutant Phage Production and Purification—The preparation of phage mutants has been described previously (14). Native and mutant phages were induced with 3 μg/ml mitomycin C from their lysogenic L. lactis 901-1 strains grown at 30 °C in GM17 broth. Following cell lysis, the phage particles were precipitated and purified by isopycnic centrifugation using a CsCl gradient.
Electron Microscopy Reconstructions of Wild-type and Mutant TP901-1 BP—For grid preparation, 3 μl of sample was applied onto a glow-discharged carbon-coated grid and incubated for 1 min. Excess solution was blotted, and 10 μl of 2% uranyl acetate was applied onto the grid. After 30 s, excess staining was blotted and the grid transferred to the microscope for data collection.

1,000 CCD images were recorded for each sample on a 4K × 4K TVIPS CCD camera using a Phillips CM200 microscope with a field emission gun operated at 200 kV (CBEM, Imperial College London) and a magnification of ×38,000 (resulting in a pixel size of 2.32 Å/pixel) under low dose conditions (supplemental Fig. S1). All data were collected over a range of nominal defocus values comprised between 0.5 and 1.5 μm. The digitized images were then coarsened by 2 × 2 pixel averaging resulting in a pixel size of 4.64 Å and their CTF corrected using IMAGIC CTF2D_FIND and CTF2D_FLIP programs. Approximately 10,000 particles were selected manually from CTF-corrected images for native TP901-1 as well as about 2,500 and 2,000 particles for bppL− and bppL+ mutants, respectively (supplemental Fig. S2). The three datasets were processed using the IMAGIC software package (25) to obtain the final three-dimensional models.

Datasets were created for the three samples by cutting the particles into 100 × 100 pixel boxes. Then, datasets were bandpass-filtered, subjected to multistatistical alignment (28), and classified with ~10 images/class. As described previously for the p2 BP (17), images corresponded to a side view orientation with the BP lying on the grid with its 6-fold symmetry axis perpendicular to the projection direction. A good class average was selected and aligned to have the 6-fold axis along the z axis. Datasets were aligned with the multireference alignment module using the aligned selected class average as reference. Subsequently, an initial model was calculated from the aligned class averages, imposing 6-fold symmetry, and re-projected along the equator (IMAGIC Euler angle β equal to 90°) with a difference of 20°. The re-projections were used for the initial angular assignment of the aligned particles using projection matching (29). Subsequent cycles of refinement including alignment, projection matching, and model calculations were iterated for 10 rounds.

The resolution of the wild-type and bppL− mutant final BP models was estimated to 25 Å by Fourier shell correlation (30) and the 1/2 bit threshold correlation criterion (supplemental Fig. S3). The resolution of the bppL+ mutant reconstruction is lower (around 28 Å) as revealed by its Fourier shell correlation (supplemental Fig. S3). Fourier shell correlation curves were obtained by correlation of two different three-dimensional models created from splitting the particles set in two halves.

Multi-angle Light Scattering Studies—Size exclusion chromatography was carried out on an Alliance 2695 HPLC system (Waters) using either a Superose 6 HR10/30 column (GE Healthcare) or a KW405-4F column (Shodex) run in a buffer containing 10 mM HEPES, 150 mM NaCl, and 0.02% NaN3 at pH 7.5 at a flow rate of 0.3 or 0.5 ml/min, respectively. Detection was performed using a three-angle light-scattering detector (MiniDAWN™ TREOS; Wyatt Technology), a quasi-elastic light-scattering instrument (Dynapro™, Wyatt Technology), and a differential refractometer (Optilab® rEX, Wyatt Technology). Molecular weight, gyration, and hydrodynamic radii determination were performed by the ASTRA V software (Wyatt Technology) using a dn/dc value of 0.185 ml/g.

Surface Plasmon Resonance—Measurements were performed at 20 °C in a buffer containing 10 mM HEPES, pH 7.5, 150 mM NaCl, 3 mM EDTA supplemented with either 0.005% or 0.05% detergent P-20 using a BLAcore X100 (GE Healthcare), respectively. We used CM5 chips (BLAcore) coated with 100 resonance units of DARPin or 1,000 resonance units of BppU-BppL, or 200 resonance units of BppL. The signal from an uncoated reference cell as well as the buffer response was subtracted from all measurements. Experiments were performed using either multiple-cycles kinetic assays or single-cycle kinetic assays. Analyte concentrations were of 10, 5, 2.5, 1.25, and 0.625 nM for DARPin or the BppU-BppL complex and 32, 16, 8, 4, 2, and 1 nM for BppL alone. The Kd, koff, and kon values were obtained using the fitting tool of the BLAevaluation software (BLAcore). A 1:1 binding model was assumed in all cases.

RESULTS

Production and Molecular Mass Determination of TP901-1 BP Individual Proteins and Complexes—TP901-1 BppU and BppL were individually produced and purified (supplemental Table S1). We also overexpressed the BppU-BppL complex using an operon-like strategy based on cloning of the DNA fragment encompassing TP901-1 bppL and bppU genes as well as the intervening sequence (13). We used a combination of static plus dynamic light scattering, UV spectrophotometry, and refractometry to characterize TP901-1 BP proteins and their complexes (supplemental Table S2). BppU and BppL measured masses were of 102,000 ± 1,000 Da and 54,000 ± 400 Da, respectively, corresponding to the trimeric form of each protein. For BppU-BppL, the measured mass was 255,000 ± 200 Da, suggesting that the complex occurred in a 3:9 ratio. Finally, overexpressing the block including ORFs 46, 48, and 49 yielded a BP with a measured mass of 1.9 MDa and hydrodynamic and gyration radii of 12.25 nm and 14.5 nm, respectively (13).

Structure of Full-length TP901-1 BppL (RBP)—We previously reported the TP901-1 BppL structure revealing its trimeric nature and in which the amino acid track is visible from residue 16 up to the C-terminal residue (8). A glycerol molecule observed in this structure led us to propose a location for the putative receptor binding site. In the present contribution, the excess BppL resulting from the operon expression of the BppU-BppL complex was used to set up crystallization trials. We obtained crystals belonging to the R32 space group and diffracting at 1.85 Å resolution, with one BppL monomer in the asymmetric unit (supplemental Table S1). The trimeric RBP is reconstituted in applying the 3-fold crystallographic symmetry. A major difference of this crystal form is that we were able to model the N terminus of the BppL chain (starting at residue 2). The 14 extra residues are in an extended conformation. This result was unexpected because the first 10 resi-
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FIGURE 1. X-ray structure of full-length BppL$_{TP901-1}$ trimer. A, two monomers are depicted in surface representation (green and blue), and the third monomer is in ribbon representation (red). Note the position of the N terminus stretch running parallel to the N-terminal helix. B, ribbon comparison of BppL$_{TP901-1}$ trimer cleaved form (PDB code 2F0C, gray) and the full-length form (pink, salmon, and yellow). The red arrow (Nt-short) points to the cleavage position in the previous structure, i.e., in the first turn of the shoulder domain α-helix (B).

dues were predicted to be structured as a α-helix. More in details, in the RBP full-length structure, residues 2–8 form an extended stretch, followed by a turn (residues 9–13) and residues 14–15 complete the helix observed in the cleaved structure (Fig. 1). Except those residues, the overall RBP structure is very similar to the previously solved one (PDB code 2F0C) with a root means square deviation value of 0.73 Å over the 148 residues in common. It should be noted that even the angle of the hinge formed between the neck and the N terminus is conserved although a slight tilt (∼1.5 Å) of the last helix is observed.

EM Reconstruction of the TP901-1 BppU-BppL complex—BppU-BppL particles adsorbed on the carbon film have a quaver-like shape showing two or more feet. Iterative particle alignment cycles followed by averaging of image classes revealed a high flexibility of this complex. From class averages analysis, different conformational states could be observed from the same orientation. Various views of two extreme BppU-BppL complex conformations were obtained, termed “packed” and “open,” differing only in the opening of the feet of the tripod, with a 20-Å feet displacement between class averages (Fig. 2A). To go further in image processing, the raw particle images were separated based on the gap between feet of the tripod. Then, we focused our analysis on the open conformation in which class-averages were better resolved yielding a reconstruction at 24.8 Å resolution.

The EM map reveals a ∼150 Å high tripod-shaped structure with a ∼120 Å wide triangular base (Fig. 2B). Each of the three tripod extensions has a height of ∼80 Å with an increasing thickness while progressing toward the tip. These extensions are plugged onto a ∼80 Å wide dome-shaped structure which exhibits a protuberance of ∼40 Å of diameter on its top. Fitting the high resolution x-ray structure of the BppL trimer into each tripod extension yielded a nice match revealing the exact BppL location in the tripod. The BppL head domain, bearing the saccharide binding site, is perfectly accommodated by the thick region of the tripod leg (Table 1 and Fig. 2C). On the opposite side, BppL N termini penetrate the dome region along a ∼10 Å distance. The remaining part of the EM density is sufficient to accommodate a BppU trimer, this hypothesis being reinforced by the good agreement between its measured mass (102,000 Da) and the volume of the dome structure.

EM Reconstructions of Wild-type and Mutants TP901-1 BP—Observation of the wild-type TP901-1 BP EM reconstruction at 25 Å resolution revealed a clear 6-fold symmetry, as expected from light-scattering solution measurements and EM studies of TP901-1 or other P335 group phages (11, 13). The BP is attached directly to the last major tail protein (MTP) ring at the end of the phage tail (Fig. 3A). Its overall dimensions are 320 Å (diameter) and 240 Å (height).

Six dome-shaped structures, with a protuberance on top, are observed in the upper part of the wild-type BP. Below, the EM map shows two elongated density cylinders, thinner close to the domes, starting from each dome region and pointing downward. A third elongation, blurred in its middle part, is observed at the most external part of the

FIGURE 2. EM of TP901-1 BppU-BppL complex negatively stained. A, selected class averages observed in various orientations and grouped in function of feet gap size, termed packed and open conformation. White arrows outline a 20-Å feet particle displacement. Each image corresponds to 360 × 360 Å. B, surface representation of the BppL-BppU three-dimensional EM reconstruction at 24.8 Å resolution. Scale bar, 25 Å. C, view of the tripod EM density with three BppL trimers fitted in the three feet. The dome and protuberance regions are attributed to the BppU trimer.

TABLE 1

| X-ray structure | EM map | CC |
|-----------------|--------|----|
| 1. 3 RBP trimers | 3BppU-9BppL tripod | 65 |
| 2. 18 RBP trimers | TP901-1 BP wild type | 39 |
| 3. SPP1 Dit dodecamer N-term | TP901-1 BP BppU mutant | 41 |
| 4. P2 ORF16 Closed trimer (from 2WZP) | TP901-1 BP BppU mutant | 35 |
| 5. Structures 3 and 4 | TP901-1 BP BppU mutant | 54 |
| 6. Structures 2, 3, and 4 | TP901-1 BP wild type | 44 |
BP. In the TP901-1 bppL<sup>−</sup> mutant, the upper domes are conserved whereas the elongated regions are absent. In the TP901-1 bppU<sup>−</sup> mutant, neither the domes nor the elongations are present (Fig. 3A). Because the dome, protuberance, and elongated densities are strikingly similar to the BppU-BppL tripod reconstruction shape, we fitted a tripod in the BP map (Fig. 3B). The top region as well as two tripod legs are perfectly accommodated in the BP map, whereas the most external (third) leg has weaker/broken density. Combining all of these results with the fitting of the BppL x-ray structure into the tripod EM map allowed us to unambiguously assign BppU and BppL positions in the BP. Three BppL trimers are located in the three elongated domains (with the head domain accommodated by the thicker part) and a BppU trimer is positioned in the dome-shaped region. It should be noted that the host-anchoring sites point away from the capsid, toward the polysaccharide cell wall. As we observed in the tripod EM structure that the BppL is plugged onto the BppU, the absence of this latter protein precludes BppL anchoring and explains its absence in the bppU<sup>−</sup> mutant. In total, we were able to position six tripods in the BP map, yielding a total of 18 RBPs (54 BppLs and receptor-binding sites) and 18 BppU molecules in the whole BP (Table 1, Fig. 4A, and supplemental Fig. S4).
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We previously determined the x-ray structure of the Dit protein (gp19.1) from phage SPP1 (31). In this structure, the N-terminal domains form two hexameric rings stacked back to back, whereas the C-terminal domains form a separate lectin-like domain located at the periphery of the rings. The SPP1 Dit fold is very similar to that of lactococcal phage p2 ORF15 (17). Sequence alignments revealed a high similarity at the level of the tape measure protein C terminus, the Dit and the Tal N terminus of many phages, including TP901-1 (31). We therefore fitted the two rings formed by the N-terminal region of SPP1 Dit (PDB code 2X8K) into two annular densities of the TP901-1 BP using either the wild-type or the bppUl− mutant reconstructions (Table 1, Fig. 4, A–C, and supplemental Fig. S4). The rings fit well, although their diameter seems slightly smaller than the EM density. We did not try to fit the SPP1 Dit C-terminal domains because the EM density does not allow unambiguous positioning. Below the Dit density, a dome-like structure is observed, with its tip in distal position. Both volume and shape of this EM density are comparable with those observed in the equivalent position in the SPP1 virion EM reconstruction (5, 31). The shape of this density is also reminiscent of the ORF16 one in phage p2 which is directly located under ORF15, a Dit homologue (17). Noteworthy, ORF16 shares a similar fold with the phage T4 gp27 (3) and the type 6 secretion system VgrG (32). We thus tentatively fitted the trimeric ORF16 structure (PDB code 2WZP) into the dome density as a model of the Tal N-terminal part. The quality of the fit is good, particularly in the bppUl− mutant where the density is clearer in this region (Table 1 and Fig. 4, A–C). To complete the pseudo-atomic model of the TP901-1 BP, we used the type 6 secretion system Hcp1 structure (PDB code 1Y12) for fitting into the MTP hexameric ring densities as it was shown to be structurally similar to the phage λMTP (33). Most EM densities could finally be assigned: BppU–ORF48, BppL(RBP)–ORF49, the N-terminal moieties of Dit–ORF46, and Tal–ORF47 (Fig. 4, A and D). We hypothesize that the Dit C-terminal domains, belonging to the upper and lower rings, anchor the BppU-BppL tripods as pincers in a way similar to the one observed in the phage p2 ORF15s to maintain the RBPs via their “arm” extensions (17). In a final step, we filled the unassigned densities of BppU–ORF48 and the extensions of Dit–ORF46 with spheres (supplemental Fig. S4). This approach made it possible to visualize more clearly the shape of Dit–ORF46 extensions and their relationship with BppU–ORF48. **Supplemental Table S3** summarizes the different names and denominations used for the various baseplate proteins discussed to facilitate reading.

**Host Anchoring Relies on Avidity**—We previously reported the successful selection of DARPinbs directed against the BppU-BppL complex. We solved the structure of one of these DARPinbs in complex with the TP901-1 BppL (RBp) and proposed the use of these binders as a biotechnological tool to fight against phage infection in the dairy industry and more generally against viruses (1, 10). While characterizing the three selected DARPinbs by BLAcore, we observed that the affinity measured between each DARPin and the tripod was dependent on the setup used. When the BppU-BppL complex was used as ligand (coated onto the CM5 chip) and DARPin20 as circulating analyte, we measured affinities in the nanomolar range (Fig. 5). In contrast, picomolar $K_a$ values were obtained when DARPinbs were used as ligand and the tripod as analyte (Fig. 5). This allowed us to conclude that DARPinbs bind to the TP901-1 BP with nanomolar affinities and that the apparent picomolar affinities result from avidity phenomena: when a DARPin is immobilized on the CM5 chip, a tripod can interact with up to three sites simultaneously (Fig. 5). When transient unbinding occurs at one site, the BppU-BppL complex cannot diffuse away, and the interaction at this site can be reestablished without diffusion. We believe that these experiments with tripod-DARPin complexes are a good model of what occurs when TP901-1 BP encounters cell wall saccharides illustrating the mechanism used by BP-bearing phages to maximize host-anchoring efficiency.

**DISCUSSION**

Phage avidity toward saccharidic receptors seems to play a major role in establishing the specificity and strength of the attachment step to the host. For example, myophage T4 probably bears 36 sites of attachment to saccharidic receptors (three sites on each of the six long and six short tail fibers) (3, 34, 35) and siphophage p2 has 18 sites (17). The present structure of phage TP901-1 BP reveals a higher number of receptor binding sites (54 in total), yet not observed in any other phage of known structure. They are grouped in six tripods of three RBPs each. A model experiment based on binding of RBP trimers to a specific DARPin revealed that the affinity of the TP901-1 tripod is ~400-fold higher (as judged by the ~400-fold $K_a$ decrease) relative to that of a single RBP trimer. No doubt that the presence of 54 sugar binding sites should lead to an irreversible binding, even if the BP is not always completely saturated and the $K_a$ for a single saccharide is closer to micromolar.

Although *Bacillus* SPP1 phage recognizes a proteinaceous receptor, its central tail tip shares common features with lactococcal phages, particularly the Dit structure. Based on sequence alignments, we propose that the N-terminal part of TP901-1 Tal might also be similar to the N-terminal domains of lactococcal phage p2 ORF16 and phage SPP1 gp21. Both Tal–ORF47 and SPP1 gp21 are large molecules of 918 and 1032 residues, respectively. It was demonstrated that the Tal C termini of Tuc2009 (and most likely of TP901-1) has a proteolytic activity, probably used to cleave the peptidoglycan and allow DNA transit into the host cytoplasm (36). Tal also exhibits an autocatalytic activity, and a consensus self-cleav-
age site was identified in Tuc2009 (GGSSG ↓ GGD) as well as in TP901-1 (14, 36, 37). In contrast, the C-terminal domain of SPP1 gp21 appears to be dedicated to receptor recognition (38). Another Tal-like protein is phage p2 ORF16. This component of the phage p2 BP is 376 residues long and seems to have only a structural role without involvement in cell wall adhesion or cleavage.

Myophage T4 and siphophage p2 exhibit an activation mechanism by which a preliminary contact with the receptors triggers a huge BP conformational change releasing the remaining receptor binding sites (3, 17). In phage p2 at rest, the adhesion or cleavage mechanism by which a preliminary contact with the receptors triggers a huge BP conformational change releasing the remaining receptor binding sites (3, 17). In phage p2 at rest, the receptors, the second as an initial trigger of the signal of DNA release because in phage p2 ORF16 exhibits an activation like effect, to all other MTP rings along the tail, finally resulting in the portal opening and DNA release. Such a mechanism was proposed, based on strong structural arguments, for siphophage SPP1 (5) and might be a general feature of Siphoviridae. However, because TP901-1 is ready for host adsorption without the necessity of a large BP conformational change, more subtle changes in the BP might occur to initiate the portal opening signal. This initial trigger could arise from BP mechanical deformations or changes in the BppLs (or tripod) orientation, resulting from their attachment to the receptors. The flexibility of the BP tripods could therefore play two functional roles, one in scanning the host surface for receptors, the second as an initial trigger of the signal of DNA release. Interestingly, such an activation mechanism based on mechanical stress was reported, in a totally unrelated system, for complement activation by the lectin pathway (39).

With the determination of several x-ray and EM structures of Siphoviridae, it becomes possible to understand their overall organization. We observe that lactococcal phages from the 936 group (e.g. p2, skl, or bIL170) or P335 group (Tuc2009 or TP901-1) exhibit common features (the BP core architecture) shared also with other phages such as SPP1. However, they exhibit very large differences in the structure and organization of the BP components which have a tremendous influence on the host recognition process, occurring with or without an activation mechanism.

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