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The portal protein of bacteriophage SPP1: a DNA pump with 13-fold symmetry

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Electron microscopy in combination with image processing is a powerful method for obtaining structural information on non-crystallized biological macromolecules at the 10-50 Å resolution level. The processing of noisy microscopical images requires advanced data processing methodologies in which one must carefully avoid the introduction of any form of bias into the data set. Using a novel multivariate statistical approach to the analysis of symmetry, we studied the structure of the bacteriophage SPP1 portal protein oligomer. This portal structure, ubiquitous in icosahedral bacteriophages which package dsDNA, is located at the site of symmetry mismatch between a 5-fold vertex of the icosahedral shell and the 6-fold symmetric (helical) tail. From previous studies such 'head-to-tail connector' structures were generally accepted to be homododecamers assembled in a 12-fold symmetric ring around a central channel. Using a new analysis methodology we have found that the phage SPP1 portal structure exhibits 13-fold cyclical symmetry: a new point group organization for oligomeric proteins. A model for the DNA packaging mechanism by 13-fold symmetric portal protein assemblies is presented which attributes a coherent functional meaning to their unusual symmetry.
Key words: bacteriophage SPP1/correspondence analysis/phage structure/protein symmetry

Introduction

Large biological macromolecules or oligomeric assemblies can be visualized easily using electron microscopy. Simple routine specimen preparation procedures, like the classic negative staining technique, often suffice to produce micrographs of non-crystallized material (single particles) suitable for structure analysis. The resolution attainable with this technique is limited to 10-50 Å, one order of magnitude lower than that obtainable by X-ray crystallography. The latter technique, however, relies on the availability of good crystals and their heavy-metal derivatives which are needed for finding both the amplitudes and the phases of the diffraction spots. It may be particularly difficult to obtain such crystals for large molecules and therefore single particle electron microscopy is often the chosen method for the initial structural characterization of large molecular assemblies.

Electron microscopy has been used extensively to characterize viruses and their substructures such as the portal protein oligomer found in icosahedral bacteriophages. This bacteriophage component, involved in several steps in phage maturation (Bazinet and King, 1985), has been previously studied by image processing of electron micrographs of purified assemblies from various bacteriophage species: T4 (Driedonks *et al.*, 1981), ϕ29 (Carrascosa *et al.*, 1982; Carazo *et al.*, 1986; Jiménez *et al.*, 1986), lambda (Kochan *et al.*, 1984), T3 (Donate *et al.*, 1988; Carrascosa *et al.*, 1990) and P22 (Bazinet *et al.*, 1988). These specimens have been analyzed either as single molecular images (Driedonks *et al.*, 1981; Bazinet *et al.*, 1988) or as 2-D crystals with apparent p6 (Carrascosa *et al.*, 1982; Kochan *et al.*, 1984; Jiménez *et al.*, 1986; Carrascosa *et al.*, 1990) or p2 (Carazo *et al.*, 1986; Donate *et al.*, 1988) symmetry. In all cases a 12-fold symmetric portal protein oligomer was found and this organization was suggested to represent a common feature necessary for their role in DNA packaging during phage assembly (Bazinet and King, 1985; Carrascosa, 1986; Casjens and Hendrix, 1988). We have recently characterized the analogous protein of the virulent *Bacillus subtilis* phage, SPP1 (gene product 6: gp6) which is essential for DNA packaging. It also plays an active role in the correct sizing of encapsidated DNA (Tavares *et al.*, 1992). In this study we present a structural analysis of this oligomer, which has an overall diameter of 175 Å and a height of 105 Å (Figure 1).

Results and discussion

One of the first techniques used to investigate electron microscopical images of single particles was the rotational power spectrum method (Crowther and Amos, 1971). This technique for finding the rotational symmetry of a molecular assembly, is based on the analysis of a single molecular image which must thus be relatively free of noise. However, the high electron exposure needed for obtaining such a noise-free image of a molecule will normally destroy the radiation-sensitive material. The alternative is to extract the symmetry information from a large number of noisy molecular images simultaneously. To find all of the original images belonging to the 'view' (projection through the assembly in a given orientation) from which the symmetry can best be determined (top view), it is necessary to first exhaustively sort all images in the data set (Van Heel and Stöffler-Meilicke, 1985). Correlation-function-based alignment techniques, however, are not suitable for conclusively finding the cyclical symmetry within the subset of top view images because alignments relative to a reference image tend to bias a data set towards the characteristics of that reference (Boekema *et al.*, 1986). In particular, alignment of a noisy data set relative to a reference image with a given circular symmetry

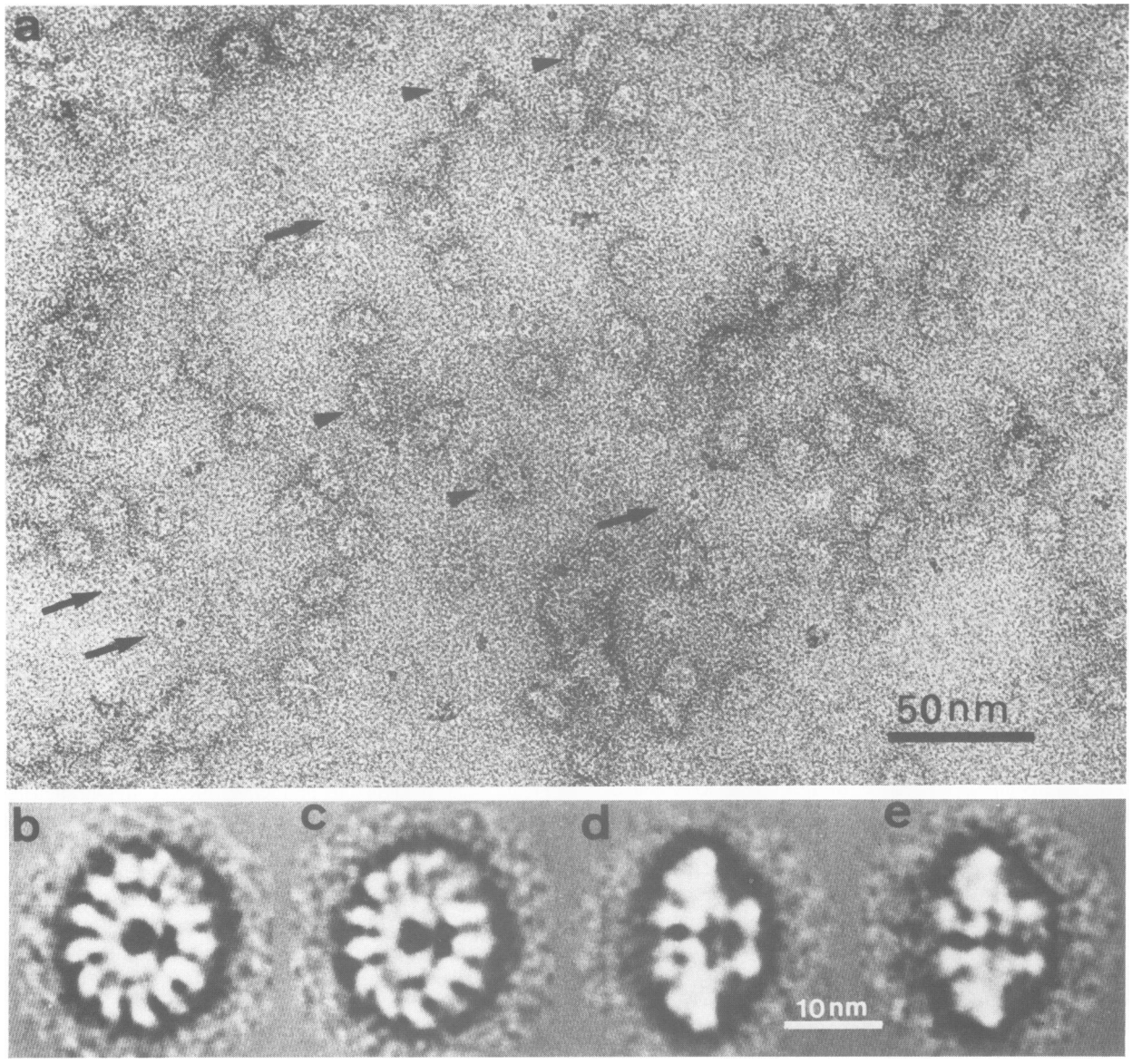

Fig. 1. A typical electron micrograph (section) of gp6 from bacteriophage SPP1 negatively stained with 0.3% uranyl acetate. The micrograph (**a**) shows the portal protein oligomers in different orientations relative to the plane of the carbon support film. Some molecules in top and side view are marked by long and short arrows respectively. Note that the stain meniscus around top views is less pronounced than those around side and tilted view orientations. From the digitized micrographs, 6200 molecular images were processed by multi-reference alignment procedures (see Materials and methods) to perform an exhaustive search of all the different orientations of the structure in the data set. Some of the resulting averaged side views (9% of the data set) and tilted views (76%) are mounted on the lower edge of the illustration (Figure 1b–e). The almost circular top views (14%) found in this part of the processing were used for symmetry analysis (Figure 2).

property may actually introduce that property into the data set; thus the argumentation risks becoming circular. To avoid such symmetry bias, only a translational alignment was used to center all the top view images relative to an artificial rotationally averaged image. Once brought to a common origin, the circular variations within this data set are assessed using eigenvector analysis aimed at finding the main sources of inter-image variations (Van Heel and Frank, 1981). The first two non-trivial eigenvectors of the data set, which themselves are images (Van Heel, 1984, 1989), will reflect the symmetry properties of the structure as identical symmetric wave patterns which are 90° out of phase (like a sine and a cosine wave). Subsequently, automatic multivariate classification procedures can be applied (Van Heel, 1989) to find the rotational orientations of the original images relative to the common symmetry pattern ('alignment by classification'). These techniques are reference-free (Schatz and Van Heel, 1990) and produce unbiased averages of the noisy microscopical images of symmetric structures.

We have applied this methodology to the analysis of purified portal protein from bacteriophage SPP1. The samples were imaged in the Suleika cryo-electron microscope (Lefranc *et al.*, 1982; Henderson *et al.*, 1990). From 30 digitized micrographs, 6200 molecular images were selected interactively and used for further image processing (Figure 1). A total of 890 top views was identified within the full data set by multi-reference alignment and classification procedures (Van Heel and Stöffler-Meilicke, 1985) and then subjected to our new symmetry analysis methodology. The resulting class averages (some shown in

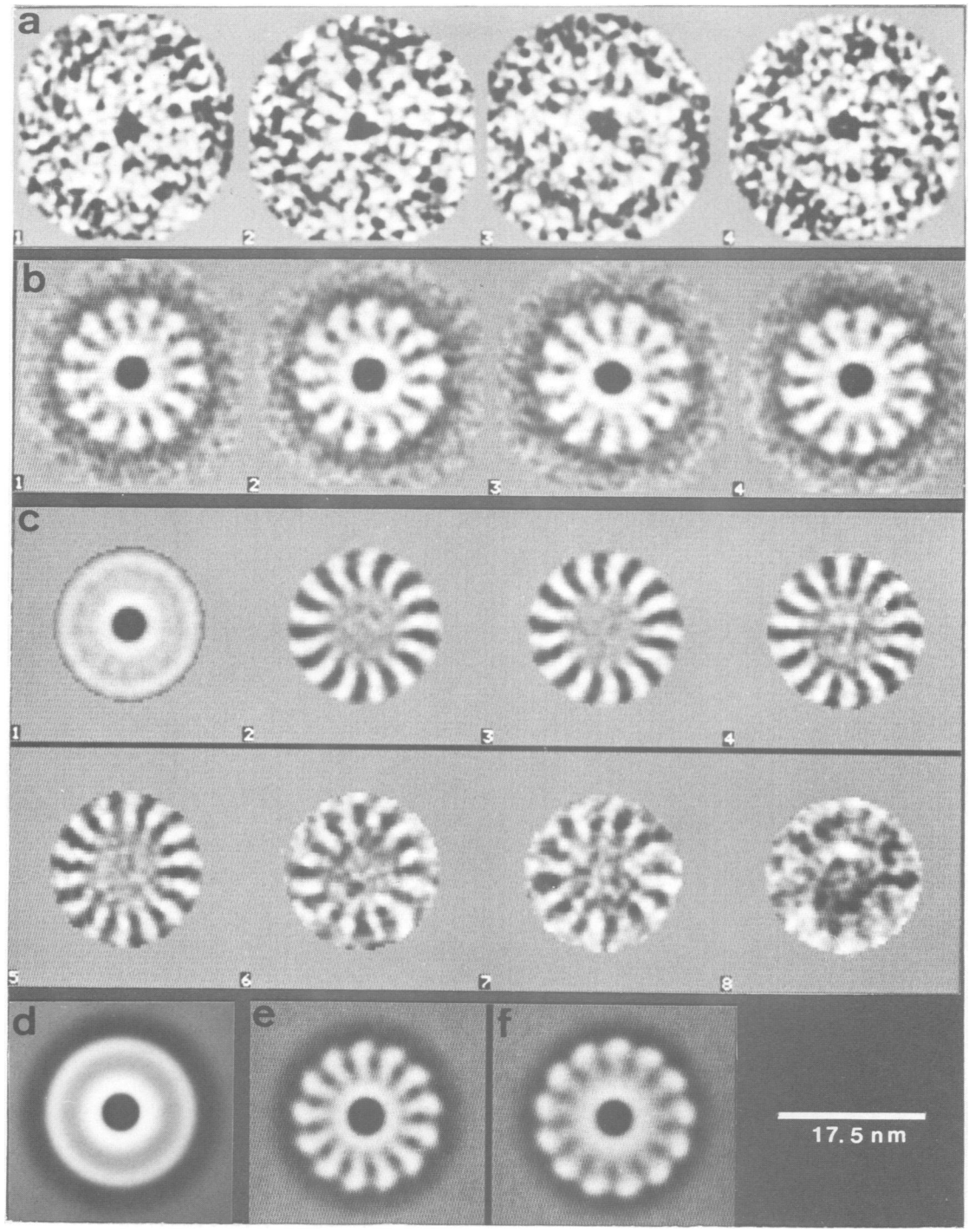

Fig. 2. Simultaneous symmetry analysis of all 890 SPP1 portal protein top view images found (Figure 1). Four (arbitrarily chosen) original images from the data set are shown in (**a**). (**b**) shows four of the 12 classes obtained after MSA classification of the top view data set. All 12 classes clearly exhibit 13-fold symmetry and differ from each other mainly by a small rotation, as can be appreciated by looking at the four averages simultaneously from the edge of the page. In (**c**), the first eight eigenimages of the analysis are depicted. The first one closely resembles the total average of this data set (Van Heel, 1989) or the rotationally symmetric mask (**d**) used for the translational alignment. The two subsequent eigenimages (nos 2 and 3) illustrate the most important differences existing within the data set: a rotational misalignment of the 13-fold symmetric component of the molecular images. Residual 14- and 12-fold harmonic components in the data set are all very weak relative to the overwhelming 13-fold component (lower by at least a factor of 7). In the final (symmetrized) SPP1 (**e**) total average, the top views have also been aligned in rotational sense. Exactly the same protocol was followed for the ϕ29 portal protein top views (1200 top views were identified from a data set of $\sim$6000 molecular images). The ϕ29 symmetrized total average (**f**) is very similar to the SPP1 end result (**e**).

Figure 2b) represent a continuum of different rotational orientations of a 13-fold symmetric structure. This organization is also reflected in the second and third eigenimages of the data set which show perfect 13-fold symmetric waves (Figure 2c; the first trivial eigenimage corresponds to just the total average). Small departures from 13-fold symmetry (tilts, see also Crowther and Amos, 1971) introduce a small 14-fold harmonic residual (eigenimage

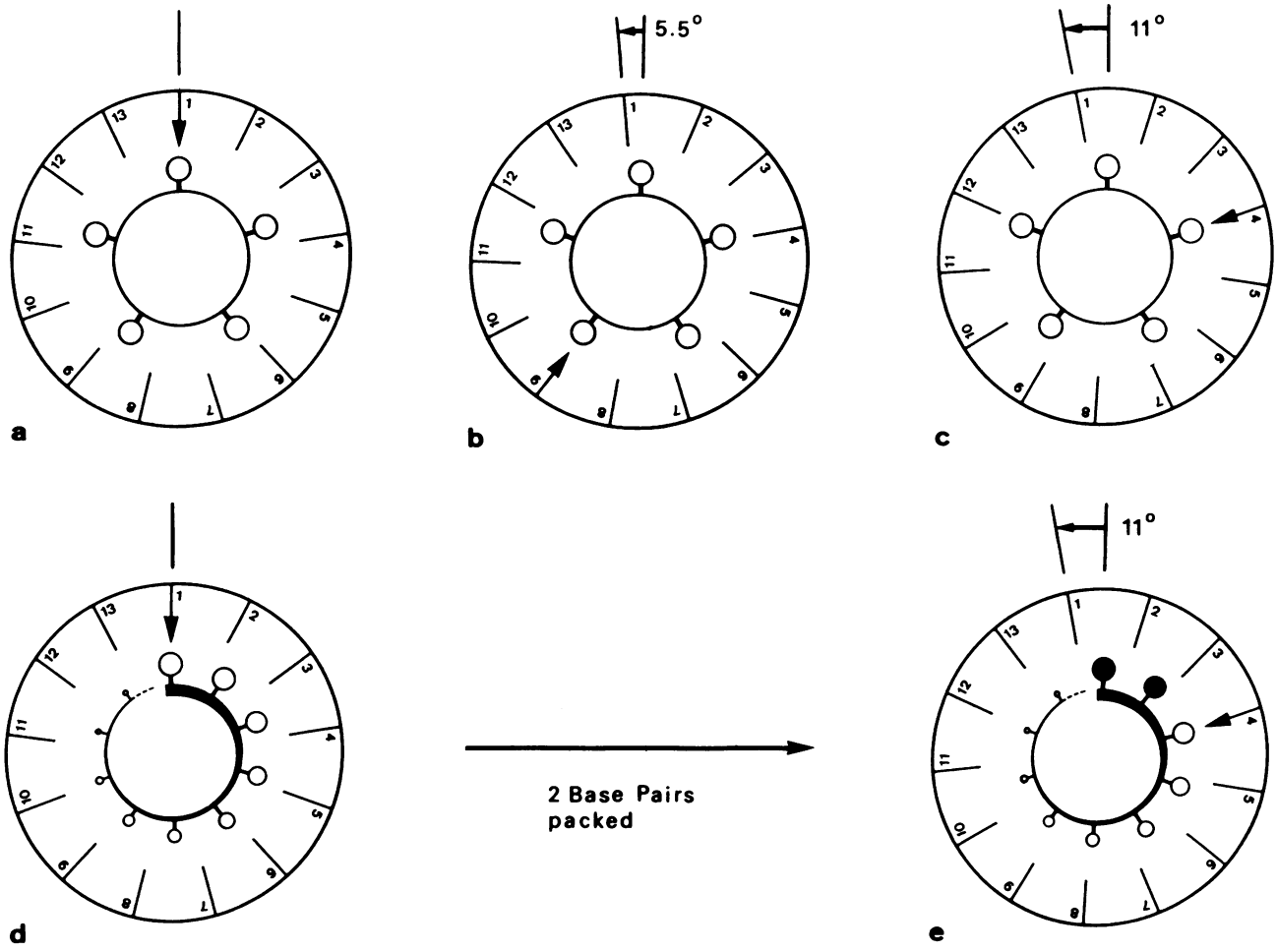

Fig. 3. (a–c) Symmetry interactions between a 13- and a 5-fold cyclically symmetric structure. A relative rotation of the two coaxial structures over a unit angle of 5.5° [=360°/(13×5)] brings the total structure into a new equivalent orientation. If the subunit 1 of the 13-fold outer circle (a) is originally in 'interaction' contact with subunit 1 of the 5-fold inner circle, one counterclockwise (positive) unit rotation of the outer circle brings subunit 9 into an equivalent contact with subunit 4 of the inner circle (b). Another such unit rotation (c) brings subunit 4 of the 13-fold circle into contact with subunit 2 of the 5-fold structure, etc. One full 360° rotation of the two structures relative to each other corresponds to 65 unit rotations. The total structure could thus well function as a molecular rotary motor consuming at least 65 ATP per 360° rotation (one or more ATP per 5.5° unit rotation). Such a rotating-'nut' driven 'bolt' pump was suggested to be the mechanism of action of DNA encapsidation (Hendrix, 1978). However, such a symmetry mismatch motor would be rather inefficient because the most probable structure to take up the bolts thread function would be the major or the minor groove of the dsDNA. One full rotation (in negative direction) of the portal protein would thus correspond to the insertion of one full 10 bp DNA helix. The energy consumption of encapsidation through this mechanism would thus be at least 6.5 ATP per bp pumped, a value 10-fold higher than the 0.5 ATP per bp determined experimentally (Guo *et al.*, 1987). An alternative mechanism based on symmetry interactions between the 10-fold helical symmetry of the DNA and the 13-fold symmetric portal protein can be described within the same formalism (**d** and **e**). For details see text and the legend of Figure 4.

numbers 4 and 5; the associated eigenvalues are ~7-fold smaller than the eigenvalues 2 and 3), and an even smaller 12-fold harmonic residual (eigenimages 6 and 7). The top views of the oligomers (see total average: Figure 2e) have a handed turbine-like appearance of 13 radial protrusions originating from a central 'tubular channel' and span an outer diameter of 175 Å. Particles with the opposite handedness were not found in the data set. This observation has been attributed to the 3-D shape of the structure (Figure 1b–e) which renders the upside-down position unstable.

The uncommon symmetry of the SPP1 portal protein prompted us to apply our technique to the analogous oligomers from bacteriophages T4 and ϕ29. A re-analysis of 2700 molecular images of the T4 assembly obtained from micrographs used in the first image processing study of this type of structure (Driedonks *et al.*, 1981) confirmed the earlier findings of 12-fold symmetry. However, in the case of the ϕ29 oligomer, a well studied protein in biological (Donate *et al.*, 1990; Donate and Carrascosa, 1991) and structural terms (Carrascosa *et al.*, 1982; Carazo *et al.*, 1986; Jiménez *et al.*, 1986), it was found that the top views (~1200 were analyzed) exhibited 13-fold symmetry (Figure 2f) in contrast to previously published results. Our work indicates that both 13- and 12-fold symmetric portal protein assemblies exist. The difficulty in detecting the tredecameric symmetry is most probably due to the image processing techniques employed in previous structural analyses of different portal proteins. These earlier studies were found to be methodologically geared towards finding even-fold symmetries: either directly towards 12-fold symmetry (single molecule analysis using the best 12-fold symmetric image as reference), 6-fold (12-fold, 18-fold,...) symmetry due to an assumed p6 crystalline arrangement, or even-fold symmetries when a p2 crystalline packing of

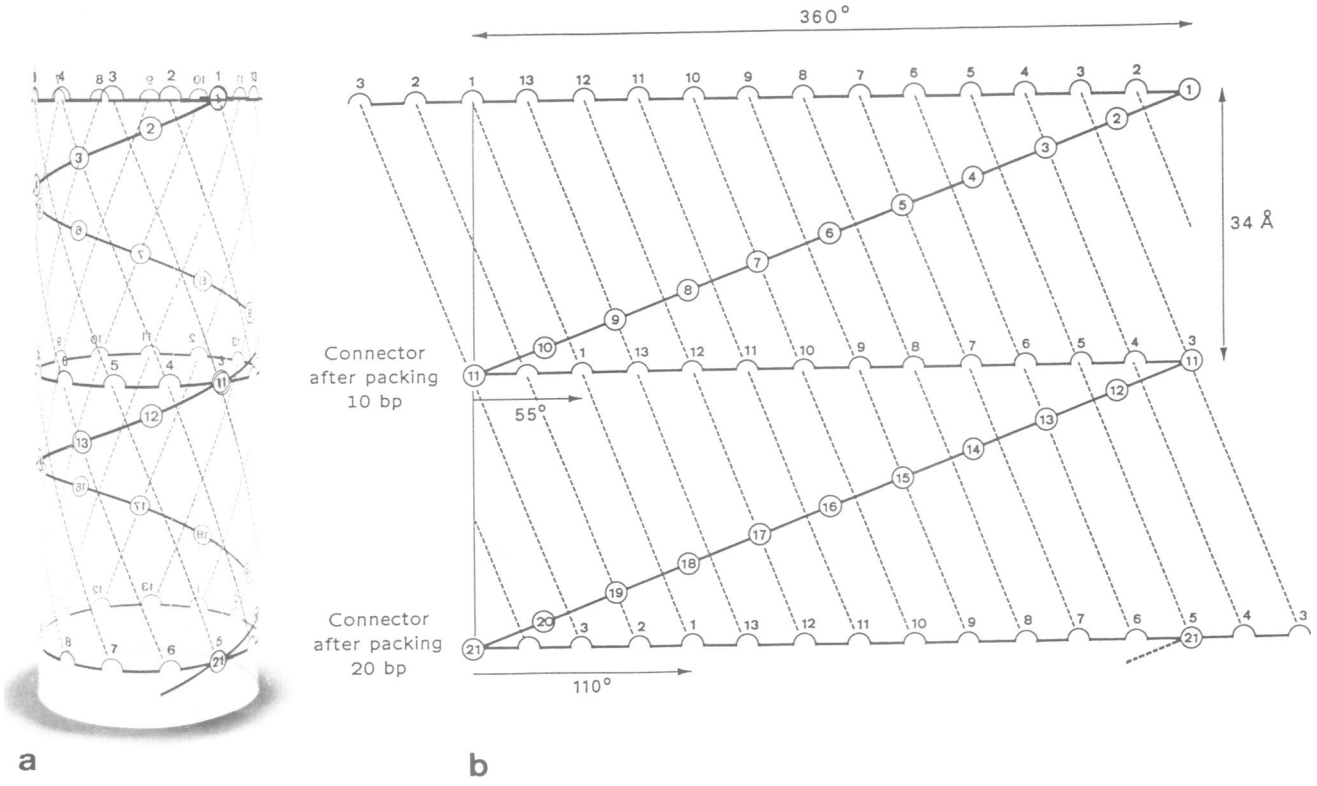

Fig. 4. Schematic diagram of the pumping action on two helical repeats of DNA (20 bp) by a 13-fold symmetric portal protein oligomer. For reasons of clarity the DNA pumping action by the portal protein is depicted as a movement of the portal protein assembly relative to a stationary DNA helix rather than the other way around. (**a**) A visual aid for understanding the cylindrical coordinates used in (b). In (**b**), the pumping over 2 bp (Figure 3d and e) corresponds to a downward shift of the portal protein (along the dotted lines) such that its subunit 4 overlaps element 3 of the DNA. The full height of (b) corresponds to the pumping of 20 bp, or two helical repeats of DNA. The rotation of the portal protein of 11° per 2 bp pumped leads to a total of 110° rotation in this figure. Note that the horizontal scale (essentially an angular scale) is chosen such that it corresponds to the linear vertical scale by placing the cylinder (a) at an effective interaction radius of 13.8 Å from the axis of the DNA. At this radius the relative movement of the portal protein subunits and the DNA is in a direction perfectly perpendicular to the DNA backbone helix, the most appealing geometric arrangement for an effective pumping action.

the portal protein was assumed. Such procedures are thus not well suited to study structures with odd symmetries.

To understand the functional meaning of the structural organization of the portal proteins, their role in dsDNA phage maturation must be considered. During this process the viral chromosome is packaged into a preassembled icosahedral prohead through the 5-fold symmetric vertex where the portal assembly is located. Following its translocation (believed to occur through the pore of the portal structure), the DNA molecule is cut and a 6-fold symmetric tail is attached to the procapsid portal vertex completing the phage morphogenesis (Casjens and Hendrix, 1988). Interestingly, the symmetries (13-/12-fold) we have found for that molecular structure do not match the 5-fold symmetry of the environment in which it is embedded (the portal vertex). This symmetry mismatch has previously been proposed to play a key role in DNA translocation (Hendrix, 1978; Casjens and Hendrix, 1988). Accordingly, a 13-fold symmetric protein rotating in a 5-fold symmetric environment could make a mechanically elegant motor in which the relative movement of the two structures, ATP hydrolysis driven, would pump the DNA into the procapsid (see legend to Figure 3 and Hendrix, 1978). Although the conceptual idea of associating rotary motor properties to a symmetry mismatch between two interacting structures is particularly interesting, the detailed mechanism proposed has to be reconsidered because the energy requirements associated with it are significantly higher than the ATP consumption determined experimentally (Figure 3; Hendrix, 1978; Guo *et al.*, 1987).

A model for DNA encapsidation consistent with all data (for a review see Hendrix, 1978; Bazinet and King, 1985; Carrascosa, 1986; Black, 1988) can be constructed which directly exploits the symmetry interactions between the 13-fold symmetric oligomer and the 10-fold helical symmetry of the DNA B conformation. Let subunit no. 1 of the portal protein assembly (Figures 3d and 4b) at a given instant interact with the first backbone element of a DNA molecule and push (direct linear to linear movement transition) the DNA helix into the capsid over the length of 2 bp. [Only for an elementary pumping action translocating 2 bp at a time were good geometric interactions found to be possible. Such action is also compatible with the energy consumption of one ATP per 2 bp (see below) found for a $\phi 29$ *in vitro* packaging system (Guo *et al.*, 1987).] This elementary pumping step brings the third DNA nucleotide pair into contact with subunit no. 4 of the oligomer (Figure 3e), provided that the oligomer has rotated over 11° (two unit rotations of $5.5° = 360°/13 \times 5$, see Figure 3a–e). The procedure can then be iterated (Figure 4). Such rotation of

the portal protein implies that the subunits move in a direction perpendicular to the edge of the double helix rather than along its major groove (Figure 4).

The rotation of the portal protein relative to the DNA being pumped is thus, in this model, not the driving force behind the translocation. The relative rotation rather serves to accommodate the reaction forces of the primary interaction between the portal subunits [or between non-structural protein(s) (terminases) transiently connected to the portal oligomer (Black, 1988)] and the DNA helix. This primary interaction force is not parallel to the DNA axis but rather perpendicular to the helical grooves on the outside of the DNA molecule. The net effect of the DNA–portal protein interaction is that the DNA is pumped straight into the capsid without any significant rotation of the dsDNA relative to the capsid being necessary. The occurrence of such rotation would lead to serious topological entanglements (Hendrix, 1978). The small topological twists required to pack the DNA in the confinement of the capsid can be compensated for at the site of the 13- to 5-fold symmetry mismatch (portal assembly versus prohead) which has a low net energy barrier for rotations (Hendrix, 1978). In this model one full 360° rotation of the portal protein corresponds to inserting 65 bp of dsDNA at the expense of a minimum of 32.5 ATPs, if it is assumed that each equivalent unitary interaction between the two structures is coupled to one ATP hydrolysis reaction and leads to the translocation of 2 bp (Figure 3d–e). Furthermore, the model predicts (Figure 4) that the portal protein subunits are activated in the order: 1, 4, 7, 10, 13, 3, 6, 9, 12, 2, 5, 8, 11, 1, etc., thereby equally employing all subunits of the portal structure during the translocation process.

With a 12-fold symmetric portal protein oligomer (Bazinet and King, 1985), the same type of DNA pumping action (also only requiring one ATP per two translocated bp of DNA) is possible. However, the steric interactions between the portal protein and the DNA are less satisfactory. The 12-fold portal protein needs three unit rotations of 6° (=360°/12×5), a total of 18°, to bring subunit no. 4 into register with the third nucleotide pair of the DNA helix after the initial interaction has occurred. The order of subunit interaction with the DNA backbone elements is then 1, 4, 7, 10, 1, etc., a circular mismatch which exempts two-thirds of the subunits from active duty. Moreover, for a 12-fold symmetric portal protein, the effective radius of interaction is 10.8 Å, as opposed to 13.8 Å for a 13-fold structure (Figure 4), leaving less room for protein–DNA interaction.

The 13-fold symmetry found for the SPP1 and ϕ29 portal structures matches well with the DNA helical symmetry in the context of the new translocation model described here. Therefore it is proposed that this is the most suitable symmetry arrangement for the portal oligomer to accomplish the DNA pumping action. The different organization found for the T4 protein may be imposed by other structural/functional requirements specific to this complex phage system.

Materials and methods

The portal protein of bacteriophage SPP1 was purified from an *Escherichia coli* overproducing strain (Tavares, 1991). In short, the overproducing cells were concentrated 30-fold in H buffer [20 mM HEPES–NaOH, pH 7.6, 10 mM EDTA, pH 8.0, 500 mM NaCl, 10% (v/v) glycerol], lysed by passing twice through a French press and the protein material was precipitated with ammonium sulfate (75% saturation). The pellet was resuspended in L buffer [20 mM HEPES–NaOH, pH 7.6, 10 mM EDTA, pH 8.0, 40 mM NaCl, 10% (v/v) glycerol] and the sample was applied to a DEAE–Sepharose Fast Flow (anion exchanger, Pharmacia) column. Gp6 was observed to elute at high salt concentrations (between 300 and 500 mM). In a final separation step, carried out in an FPLC system (Pharmacia), the semi-purified material was applied to a Mono Q column (Pharmacia) and eluted with a programmed two-step 40–500 mM NaCl gradient. The purified protein species was identified as gp6 by several criteria (Tavares, 1991 and our unpublished results).

The gp6 sample was loaded on fresh glow-discharged, carbon coated grids and stained with 0.3% uranyl acetate. All electron images were recorded using a Suleika 4 K cryo-electron microscope (Lefranc *et al.*, 1982; Henderson *et al.*, 1990) to minimize radiation damage and stain crystallization during the exposure to electrons. The specimen temperature in the microscope was ~5 K. All micrographs were taken at a magnification of 66 000×, an acceleration voltage of 100 kV and a defocus range from −1100 to −9000 Å (first zero of the ctf = 1/5.5–1/18 Å). The total electron dose used for searching and imaging was ~45 e^{-}Å^{-2} which, due to a cryoprotection factor of ~10, corresponds to ~5 e^{-}Å^{-2} at room temperature (Zemlin *et al.*, 1986). Thirty micrographs were digitized using a Datacopy 610F CCD densitometer with a sampling size of 0.38 nm at the specimen scale. All image analysis was performed using the IMAGIC-5 software system (Van Heel and Keegstra, 1981) on VAX-3100 workstations and IBM-PC computers. From the digitized micrographs 6200 molecular images (each 72×72 pixels) were selected interactively. All separate molecular images were used to obtain an unbiased statistical representation in the data set of the different views present in the micrographs. The molecular images were band-pass filtered (spatial frequencies < 1/135 Å and > 1/9 Å were suppressed), normalized and then aligned by iterative multi-reference alignment procedures (Van Heel and Stöffler-Meilicke, 1985). Multivariate statistical analysis (MSA) eigenvector eigenvalue data compression (Van Heel and Frank, 1981) and classification methods (Van Heel, 1984, 1989) were used to separate the different molecular views (different orientations of the protein relative to the support film) present in the image data set. All class averages shown in this paper have a resolution of ~20 Å as determined by the S-image criterion (Sass *et al.*, 1989). About 76% of the images represent various tilted orientations (Figure 1b–c) and ~9% are mushroom-like side views (Figure 1d–e) of the molecule. The most interesting views for this study are the almost circular top views (14% of the total image data set: 890 individual molecular images) with which the symmetry properties of the oligomers are studied (Figure 2a–b).

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