the lipid chains and with the hydrophilic side exposed to the lipid headgroups and solvent. The two helices are connected by a mobile loop and residues at both the NH_2- and COOH-termini of the protein are mobile. The structures of both photosynthetic reaction centers (*13*) and bacteriorhodopsin (*14*) are dominated by long hydrophobic membrane-spanning helices connected by loops, some of which contain relatively short amphipathic bridging helices. Bacteriorhodopsin has been shown to have mobile NH_2- and COOH-terminal regions as well as mobile internal loops (*15*). The dynamics of the coat protein from a different filamentous bacteriophage (fd and M13) has also been characterized in some detail, showing that it also has mobile NH_2- and COOH-terminal regions in its membrane-bound form (*16–18*). The mobile NH_2- and COOH-terminal regions as well as the mobile internal connecting loops are characteristic of membrane-bound proteins, as are the rigid hydrophobic bilayer spanning helices and amphiphatic bridging helices. Pf1 coat protein is a typical membrane protein.

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Membrane-Mediated Assembly of Filamentous Bacteriophage Pf1 Coat Protein

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Filamentous bacteriophage Pf1 assembles by a membrane-mediated process during which the viral DNA is secreted through the membrane while being encapsulated by the major coat protein. Neutron diffraction studies showed that in the virus most of the coat protein consists of two α-helical segments arranged end-to-end with an intervening mobile surface loop. Nuclear magnetic resonance studies of the coat protein in the membrane-bound form have shown that the secondary structure is essentially identical to that in the intact virus. A comparison indicates that during membrane-mediated viral assembly, while the secondary structure of the coat protein is largely conserved, its tertiary structure changes substantially.

THE COAT PROTEINS OF FILAMENTOUS bacteriophage are among the simplest examples of transmembrane proteins. Prior to assembly they are inserted into the inner host cell membrane with their NH_2-termini on the outside of the membrane and their COOH-termini on the inside (*1, 2*). During assembly, the basic amino acids near the COOH-terminus of the coat protein form electrostatic interactions with the DNA (*3*) and the proteins interact with one another to form the long (1 to 2 μm), flexible, 65 Å diameter virions with no incorporation of lipid. Characterization of the process by which the protein coat assembles around the viral DNA as it is secreted through the host cell membrane may aid in our understanding of a number of membrane-mediated processes, including assembly of membrane components and protein secretion. In this report we compare the structure of the coat protein of filamentous bacteriophage Pf1 in the intact virus particle [as determined by neutron diffraction (*4, 5*)] with its membrane-bound conformation [as determined by nuclear magnetic resonance (NMR) (*6*)]. This comparison provides information about the structural transformations that take place during membrane-mediated translocation processes.

Neutron diffraction from magnetically oriented gels of specifically deuterated virus particles has been used to determine the positions of 15 of the 46 amino acid residues in the coat protein of filamentous bacteriophage Pf1 (*4, 5*). A typical neutron fiber diffraction pattern from a native (unlabeled) specimen is shown in Fig. 1. Difference

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Table 1. Secondary structure of the Pf1 coat protein. The intact virion results are from neutron diffraction. Results on the coat protein in membranes are from NMR spectroscopy. Solid-state NMR spectroscopy shows that Gly^{15} or Gly^{17} or both are mobile in the surface loop of the virus.

Intact virion		Membrane	
Residues	Structure	Residues	Structure
		1–5	Mobile
2–12	α Helix	6–13	α Helix
13–19	Surface loop	14–18	Mobile
20–42	α Helix	19–42	α Helix
43–46	No information	43–46	Mobile

maps between deuterium-labeled and unlabeled phage particles were produced with the use of phases from rod models initially developed for interpretation of x-ray diffraction data from Pf1 (7) and refined against the neutron diffraction data (4).

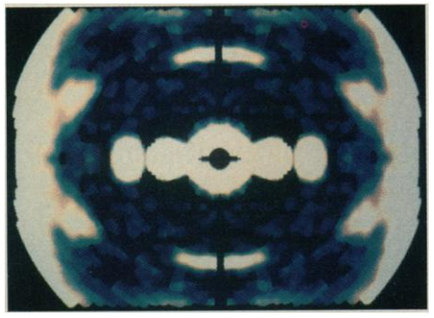

Fig. 1. Diffraction pattern from an oriented gel of native filamentous bacteriophage Pf1 taken with two-dimensional (2-D) detector at the High Flux Beam Reactor (Station H3A) at Brookhaven National Laboratory. Specifically deuterated virus particles were produced by growing the virus in minimal media in the presence of deuterated amino acids. Experiments were carried out with labeled valine (five residues per protein), isoleucine (six per protein), methionine (two per protein), and tyrosine (two per protein). The virus particles were oriented for diffraction experiments by placing a 20- to 40-mg/ml solution in a 5.8-T magnetic field for 12 to 24 hours. Orientation was preserved on removal from the magnetic field by orienting the particles in the presence of acrylamide and polymerizing the acrylamide around the particles once they were oriented but before they were removed from the magnetic field. Diffraction experiments were carried out with the specimens in 100% D_2O to minimize background due to inelastic scattering from the solvent. The H3A station is designed for crystallographic applications and is equipped with a multiwire 2-D area detector (*12*). Diffraction data collected in this way were corrected for disorientation, beam size, and other geometric distortions with the use of a 2-D deconvolution procedure (*13*).

The interpretation of the difference maps is based on the high α-helical content of the coat protein. Results of x-ray fiber diffraction (*7, 8*) and solid-state NMR studies (*9*) of Pf1 both indicate a high proportion of α-helical structure, with the α helices oriented nearly parallel to the viral axis. A wide variety of other spectroscopic studies are also consistent with a largely α-helical structure for the Pf1 coat protein (*10*). Based on these results, we constructed α-helical models for the coat protein consistent with the experimentally derived positions of the deuterium labels.

The positions of the 15 labels as determined from the difference maps for the specimens labeled with Val, Ile, Met, and Tyr are shown in Fig. 2A. Superimposed on these positions is the best fit of two α helices to the label positions. One helix was fit to the positions of amino acid residues 2, 3, 8, and 12; the other to the positions of residues 22, 25, 26, 27, 31, 32, 35, 39, 40, and 42. The position of Met^{19} is immediately

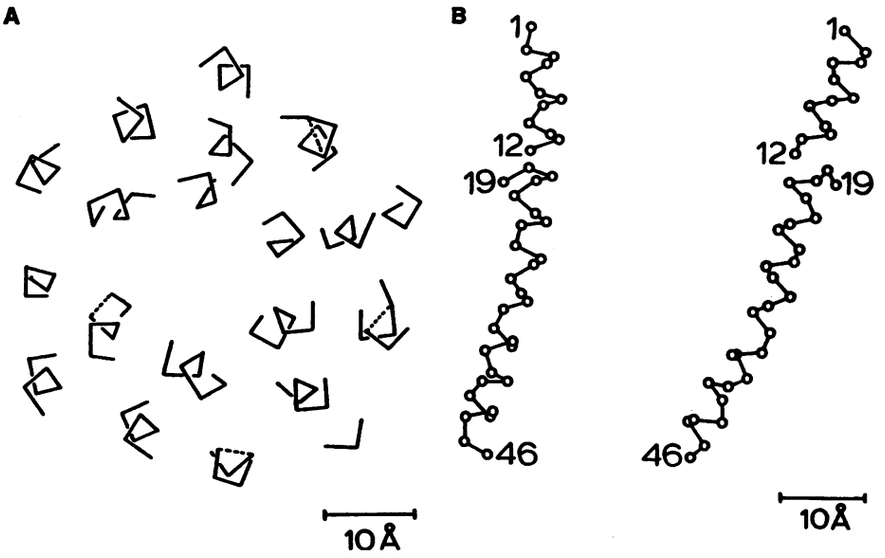

Fig. 3. Positions of the α-carbons in the pair of α helices making up the model for the structure of the Pf1 coat protein. (**A**) A 10 Å thick cross section of the bacteriophage. The nonhelical loop is not shown here. The positions of Ile^{12} and Met^{19} are indicated by a broken line connecting them. They appear near the surface of the virus particle, near the bottom of a small depression that may hold the nonhelical loop. (**B**) A pair of orthogonal views of the model for the Pf1 coat protein. These views allow the continuous structure of the α helices to be seen clearly.

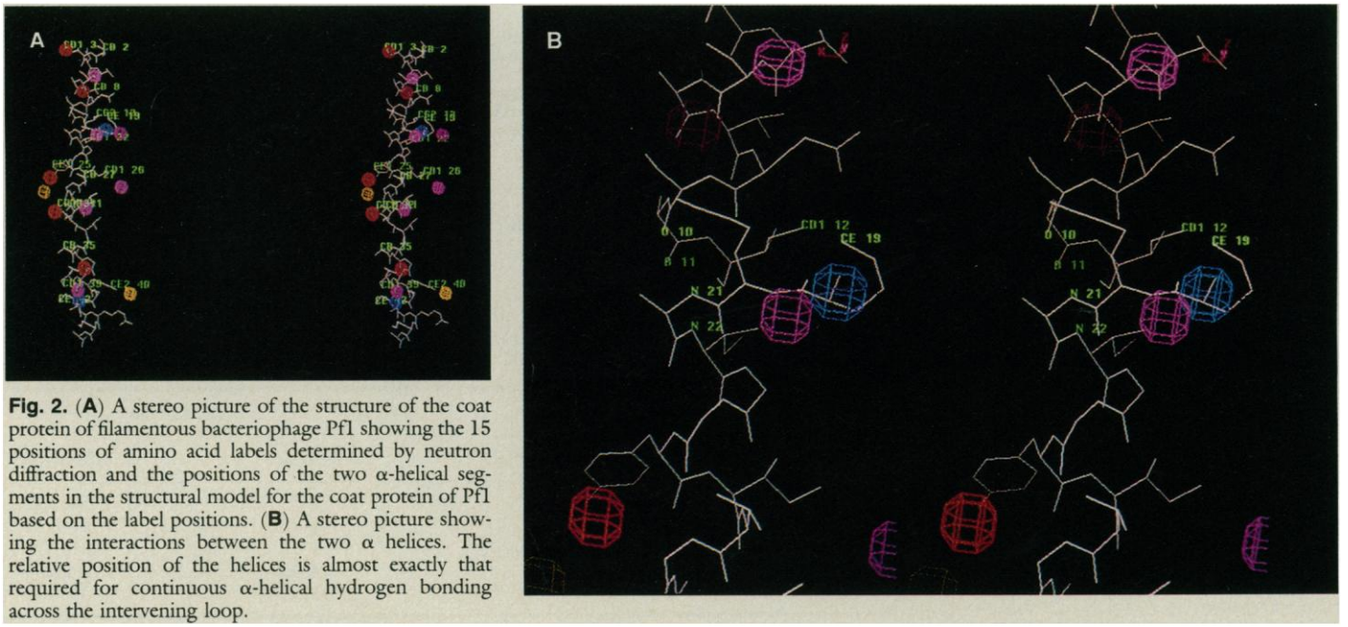

Fig. 2. (**A**) A stereo picture of the structure of the coat protein of filamentous bacteriophage Pf1 showing the 15 positions of amino acid labels determined by neutron diffraction and the positions of the two α-helical segments in the structural model for the coat protein of Pf1 based on the label positions. (**B**) A stereo picture showing the interactions between the two α helices. The relative position of the helices is almost exactly that required for continuous α-helical hydrogen bonding across the intervening loop.

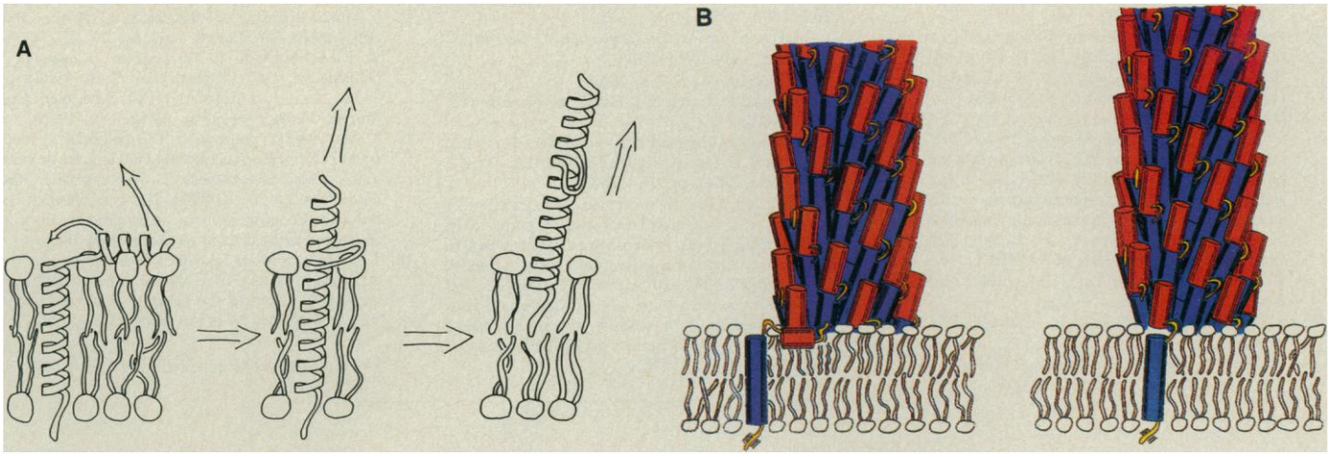

Fig. 4. Model for the structural changes that occur in the Pf1 coat protein during viral assembly. The coat protein is initially an integral membrane protein in the host cell membrane. The COOH-terminal α helix spans the membrane with the positively charged Arg^{44} and Lys^{45} on the inner surface. The NH_2-terminal α helix is on the outside of the membrane, lying parallel to the surface of the membrane. The intervening loop is mobile. Assembly of the protein into the virus may initiate when the protein interacts with the growing viral helix at the external membrane surface, or when the COOH-terminal positive charges interact with the DNA at the inner surface of the membrane, or when both of these interactions take place. During assembly, the two α helices rearrange themselves relative to one another to form the continuous helical structure. As this occurs, the protein is pushed out through the membrane, the nonhelical loop arranging itself on the viral surface. (**A**) The coat protein is drawn as a ribbon diagram shown relative to the host cell membrane without showing other components of the virus. (**B**) The growing cylinder of the virus is shown relative to the assembling coat protein to provide a hypothetical view of the membrane-associated viral assembly and protein structural changes. In this drawing, each α helix is drawn as a cylinder. The intervening loop is shown as a flexible connection between the two α helices, but its exact location has not been determined.

adjacent to the ends of the two helices but does not fit well into either one. A substantial improvement in the fit was obtained by adding a slight bend in the long helix between Ile^{32} and Val^{35}. The average distance between the experimentally determined label positions and the corresponding positions in the α-helical model is 4.1 Å. This discrepancy should decrease on refinement of the molecular model.

The two helices abut end to end to form a helical structure with an approximate 15° bend between Ile^{12} and Lys^{20} and excluding residues 13 to 19 (Fig. 2B). This type of structure is not without precedent (*11*). Considering the packing of protein in Pf1 and the position of Met^{19}, the seven excluded residues appear to form a mobile loop located adjacent to the NH_2-terminus of a symmetrically equivalent protein. Figure 3A shows a 10 Å thick cross section of the α-helical model for the Pf1 coat protein in which the positions of α carbons are used to show the packing of helical segments in the virus. Figure 3B shows a pair of orthogonal views of a single protein showing the continuous α-helical conformation across the bend in the helix caused by the exclusion of residues 13 to 19.

A comparison of the secondary structure of the coat protein of Pf1 in the intact virus with that in its membrane-bound form (*6*) (Table 1) shows that the secondary structure in the two environments is similar. In the membrane, the NH_2-terminal amphipathic α helix extends from Ser^{6} to Thr^{13} and the long hydrophobic helix extends from Met^{19} to Met^{42}. In forming the virus several small structural changes occur. The NH_2-terminus, which is flexible in the membrane, adopts a defined conformation in the virus. The loop from Gly^{15} to Gly^{17} is flexible both in the membrane and in the virus (*6*). The interhelical hydrogen bonds that connect the two abutting helices form during viral assembly. The COOH-terminal residues, which are flexible in the membrane, interact with the DNA in the intact virus (*3*). Neutron data are consistent with these residues having an α-helical structure in the virus, but are not adequate to prove it.

The results provide the basis for a model of the conformational changes that take place in the coat protein during the viral assembly. Initially, the coat protein exists as an integral membrane protein spanning the host cell membrane with its NH_2-terminus outside and its COOH-terminus inside (Fig. 4A). Since the NH_2-terminal helix is amphipathic and the NMR data show it to be parallel to the plane of the lipid bilayer, it may sit on the surface of the membrane with its hydrophobic residues in contact with the lipid hydrocarbons. The stretch of 23 hydrophobic residues beyond the loop (Met^{19} to Leu^{43}) is sufficient to span the membrane, with the positively charged COOH-terminal residues (Arg^{44} and Lys^{45}) exposed on the inner surface of the membrane. The residues forming the loop between the helices are flexible, as indicated by the NMR results (*6*) summarized in Table 1. During assembly, the NH_2-terminal helix must become almost perpendicular to the membrane surface and form the hydrogen bonds that connect the two helices end to end. The seven residues excluded from the helical structure then form a surface loop on the virus. This process is diagrammed in Fig. 4B.

Comparison of the structure of the coat protein of filamentous bacteriophage Pf1 in the intact virus as determined by neutron diffraction, and in membranes as determined by solid-state and two-dimensional NMR, has provided the most detailed structural information yet available for a membrane-associated assembly process. The recycling of viral coat proteins in cells infected by the phage (*2*) implies that this process is reversed during the insertion of the coat protein into the membrane. Consequently, the interhelical hydrogen bonds are broken during the insertion of the protein into the membrane during viral disassembly. These results indicate that the secondary structure of the Pf1 coat protein is conserved during viral assembly, but that the protein tertiary structure (the spatial relations and bonds between the secondary structures) changes substantially in the transition from the environment of the membrane to that of the intact virion. Conservation of secondary structures coupled with changes in tertiary structure may be a common characteristic of assembly pathways used during the insertion of proteins into membranes or translocation of proteins through membranes.

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14. This work could not have been done without the very generous assistance of B. Schoenborn, who helped with every aspect of the neutron diffraction experiments. Supported by grants from NIH and NSF.

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Separation of IL-4 Production from Th Cell Proliferation by an Altered T Cell Receptor Ligand

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In the presence of antigen presenting cells, a murine T helper (Th) cell specific for murine hemoglobin (Hb) responded to its immunogenic peptide by both cytokine (interleukin-4) secretion and proliferation. An altered Hb peptide with a single amino acid substitution induced only cytokine secretion and did not induce proliferation. Interleukin-1 costimulated and restored the Th proliferative response to normal levels. The altered peptide also supported cognate T cell–B cell interactions indicative of T cell helper function. Thus, this result suggests that the T cell receptor has the capacity of differential signaling.

THE COMBINATION OF A PROCESSED peptide and a major histocompatibility complex (MHC) molecule comprises the ligand for the T cell receptor (TCR) (*1*). After recognition of peptide-MHC complexes, the TCR transduces signals, resulting in the production of cytokines and in the entry of the cell into a proliferative cycle (*2*). As secreted cytokines execute many of the functions ascribed to $CD4^+$ Th cells, a proliferative response of the Th cell may be unnecessary for an immune response (*3–6*). To examine whether T cell cytokine production inevitably leads to proliferation, we have used a cloned Th cell. Unfortunately, cloned Th cells are selected for their in vitro proliferative response to antigen. To circumvent this problem, we systematically replaced amino acids in an immunogenic peptide, and these altered TCR ligands were then tested for Th cell activation. The change of a single amino acid modifies the TCR ligand so that cytokine production disassociates from proliferation.

Murine $CD4^+$ T cells that react to the β^{dminor} chain of murine Hb respond to the peptide fragment containing amino acids 64 to 76 (*7, 8*). The T helper clone (2.102) used in this study secreted interleukin-4 (IL-4) but not IL-2 or interferon-γ after stimulation and so displays the T helper 2 (Th2) phenotype (*9, 10*). The 2.102 cells proliferated in response to the Hb(64-76) peptide and antigen presenting cells (APCs) (Fig. 1A). When single, conservative amino acid substitutions were introduced into the peptide at positions 69 through 76, a range of effects on proliferation were observed (Table 1). Substituted peptides such as Gln72–Hb(64-76) and Asp73–Hb(64-76) were unable to induce a proliferative response at peptide concentrations as high as 3200 μM, which is equivalent to 4.7 mg of peptide per milliliter (Fig. 1A). Most conservative amino acid substitutions do not affect the binding of MHC molecules and immunogenic peptides (*11*). All of the substituted peptides used in this study bound to MHC $I\text{-}E^k$ molecules in both a functional competition assay and direct binding analysis (*12*).

As an alternative assay for T cell activation, the secretion of IL-4 was measured after stimulation of 2.102 cells with the Hb peptides and APCs (Fig. 1B). In agreement with its inability to support a T cell proliferative response, the Gln72–Hb(64-76) peptide was unable to induce IL-4 production. In contrast, the substitution of Asp for Glu at position 73 stimulated cytokine production (Fig. 1B), even though the substitution was incapable of inducing Th cell proliferation. A small shift (tenfold) was seen in the dose curve generated in response to the Asp73–Hb(64-76) peptide. Peptides that caused similar shifts in the cytokine curve such as Arg76–Hb(64-76) caused Th cell proliferation (Fig. 1A). Although it is difficult to quantitate the minimum amount of IL-4 needed for Th cell proliferation, 100 μM of the Hb(64-76) or the substituted Asp73–Hb(64-76) peptide induced similar concentrations of IL-4, and the addition of exogenous IL-4 [recombinant IL-4 (rIL-4) 250 units/ml or an IL-4 containing supernatant >2000 units/ml] did not result in any proliferation of the Asp73–Hb(64-76)– stimulated cells (*12*). Thus, the lack of proliferation of the Asp73–Hb(64-76)–stimulated cells was not due to the manufacture by the T cells of insufficient IL-4 for autocrine growth. This conclusion is also supported by the observation that the supernatants were collected after 24 hours, at which time there was only minimal T cell proliferation and the T cells would have potentially utilized only a small amount of IL-4.

In MHC-restricted T cell–B cell collaboration, B cell activation of Th cells results in the transmission of an unidentified cell-cell signal that enables the B cell to respond to cytokine (*13, 14*). As an assay for cognate T cell–B cell interaction, the panel of substituted Hb(64-76) peptides and the 2.102 cells were used to stimulate B cells. B cell proliferative responses correlated with the production of cytokine by the Th cells (Table 1). The Ser72–Hb(64-76) peptide did not activate IL-4 secretion by the Th cells and failed to cause B cell proliferation, whereas the Asp73–Hb(64-76) peptide supported a B cell proliferative response, inducing Th cell cytokine production in the absence of Th cell proliferation (Fig. 2A). The polyclonal production of immunoglob-

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