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ASSEMBLY AND ATTACHMENT OF BACTERIOPHAGE T4 TAIL FIBERS

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Bacteriophage T4 tail fibers are rodlike structures with a contour length of about 1400 Å, a diameter of about 45 Å, and a total mass of about 600,000 daltons. The assembly of the tail fibers and their subsequent attachment to the phage particle are under the control of 8 phage-induced proteins. The gene control and molecular weight of each protein are known. The sequence of gene-controlled steps has been determined by the characterization of intermediates that accumulate when various steps are blocked by mutation. The protein composition of the fibers and their precursors has been determined by purification and electrophoretic analysis.

Four of the eight gene products are structural components of the tail fiber. These proteins are P34 (150,000 daltons, 2 copies), P37 (120,000 daltons, 2 copies), P35 (40,000 daltons, 1 copy), and P36 (24,000 daltons, 2 copies). The wac (whisker antigen control) gene product is a structural component of the phage whiskers. The remaining three gene products, P38, P57, and P63, are not structural components of the phage particle. Both P63 and the wac gene product promote the attachment of tail fibers to the phage particle. P63 has been shown to act catalytically. Both P38 and P57 are somehow involved in the folding of the major tail fiber structural proteins (P37 and P34). The normal requirement for P38 and P57 functions can be bypassed by secondary mutations.

Bacteriophage T4 tail fibers provide a convenient model for studying the assembly of a supramolecular protein structure. Since the assembly and structure of T4 tail fibers has been reviewed recently (1), we shall confine our discussion primarily to the roles of nonstructural, phage-coded proteins in tail fiber assembly and attachment. We shall also describe recent experiments demonstrating that a non-tail-fiber structural protein facilitates the attachment of tail fibers to fiberless phage particles.

THE TAIL FIBER ASSEMBLY PATHWAY

The pathway for T4 tail fiber assembly has been characterized in some detail (for

review see 1). The protein products of the phage genes that control fiber assembly have been identified electrophoretically; the pathway intermediates all have been purified and characterized, and all but the initial assembly steps have been demonstrated in vitro. Six phage gene products are required for assembly of the fiber, but only four of these are found in the completed structure or its precursors.

Figure 1 is a schematic representation of the tail fiber assembly pathway, as established by in vitro studies and electron microscopy (2, 3). The steps take place in a defined sequence that is determined at the level of protein–protein interaction. The tail fiber is composed of two rodlike half fibers which are assembled independently. The proximal, or A half fiber, is a rod-shaped dimer of a single protein species, the product of gene 34 (P34). The monomer of P34 has a molecular weight of about 150,000 (4, 5). P57, a nonstructural phage-coded protein, facilitates the dimerization of P34. We shall discuss the function of P57 in more detail below.

Assembly of the distal, or BC′ half fiber, requires the function of 5 phage gene products (2). In the initial step, two P37 molecules (molecular weight 120,000), the major structural components of the BC′ half fiber, dimerize to form the rod-shaped C half fiber (4). This step requires the function of two nonstructural phage-coded proteins, P38 and P57. The next step in the pathway is the addition of two molecules of P36 (molecular weight 24,000) to the proximal end of the C half fiber (5, 6). The addition of P36 is associated with an increase in length from about 560 Å to about 690 Å and the appearance of the B antigen (3). The last protein added to the distal half fiber is a single copy of P35 (molecular weight 40,000) (6). P35 is required for the association of the BC′ and A half fibers. No measurable increase in length or new antigen accompany the additior of P35 (3).

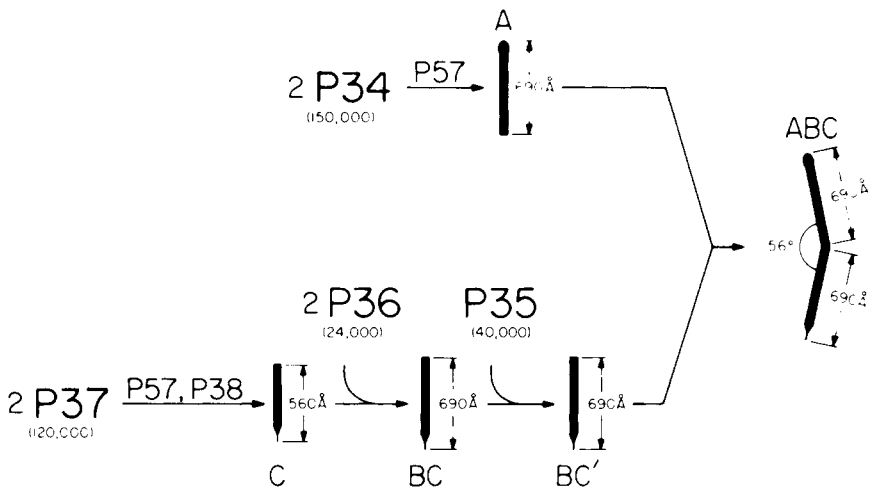

Fig. 1. The sequence of steps in T4 tail fiber assembly. Numbers preceded by P represent the protein products controlled by numbered genes. Dimensions and appearance of structural intermediates are based on electron microscopy, and antigens A, B, and C are determined by serum blocking assay (3). The molecular weights of the four structural proteins are indicated in parentheses (4–6).

DIMERIZATION OF P37

The two P37 molecules are arranged approximately linearly and in parallel in the half fiber, with the N-terminal ends at the central kink of the whole fiber and the C-terminal ends at the distal tip. This arrangement was determined by observing the positions at which antibodies to specific portions of the polypeptide chain bind to complete tail fibers (7). The evidence is consistent with a regular, perhaps double-helical, configuration of the two P37 molecules in the C half fiber with an average translation along the fiber axis per residue of about 0.5 Å in the thick region, and about 0.9 Å per residue in the thin distal extension. The assembly of this structure in vivo from P37 monomers requires the functions of P38 and P57.

P38

P38 has been identified on SDS-polyacrylamide gels and shown to have a molecular weight of 26,000 (5, 6). This protein is not associated with either the completed fiber or any of its precursors (6). P38 activity can be assayed in vitro, but its instability in crude extracts has hindered purification and characterization of the active protein. Nevertheless, we have determined that the reaction requires no dialyzable cofactors, and that the rate is temperature-dependent with no detectable activity at 4°C.

Genetic experiments have provided further insight into P38 function. These experiments were directed at the question of whether the C antigen that appears as a consequence of P38 activity results from alteration of the covalent structure of P37 (e.g., cleavage, hydroxylation, etc.) or simply from a conformational change of P37 that is facilitated by P38. The isolation of mutant phage that could bypass the requirement for P38 (i.e., pseudorevertants of 38-defective phage) would argue against the first possibility and for the second. Several such mutants have now been found (1, and unpublished observations). The best characterized of these carries a secondary mutation in gene 37 that allows growth in the absence of P38 function. The pseudorevertant produces about 20% the normal level of BC antigen and about one-half the normal level of progeny phage. This observation suggest that the step normally facilitated by P38 does not involve major alteration of P37 covalent structure.

P57

Like P38, P57 is essential for formation of C half fibers, but it is also required less stringently for the production of A half fibers and for the incorporation of P12 into the phage baseplate (3, 5, 8). P57 has a molecular weight of about 10,000 daltons, and like P38 is not found in either the completed phage or in any assembly intermediates. P57 is the only protein involved in fiber assembly that cannot yet be assayed in vitro. However, as with P38, a genetic approach has provided some insight into its function.

In the absence of P57 function, P37, P34, and P12 are synthesized in normal amounts, but only 5–10% of the normal level of C antigen and 10–40% of the normal level of A antigen are produced (4, 5, 9). These antigen levels vary somewhat in different

bacterial host strains. This observation, as well as the pleiotropic effect of gene 57 mutations, led us to suspect that P57 function might involve some general alteration of the host cell that is more essential in some hosts than in others. This idea was strongly supported by the isolation of host mutants that specifically allow T4 to grow in the absence of P57 function (1). These mutants, which are now being characterized, should provide further insight into the nature of P57 function.

In summary, P37 monomers normally cannot associate spontaneously into the configuration they assume in the half fiber without the function of two additional nonstructural proteins. The requirement for either of these proteins can be bypassed by mutational alteration of P37 in the case of the P38 requirement and by mutation of the bacterial host in the case of the P57 requirement.

ATTACHMENT OF TAIL FIBERS TO THE PAHGE BASEPLATE

Information concerning the role of phage-coded proteins in tail fiber attachment comes primarily from in vitro experiments. Attachment can be assayed by measuring the appearance of infectious virus in a mixture containing fiberless particles (produced using a tail fiber-defective mutant) and tail fibers (produced using a mutant defective in head and tail formation) (10). Two phage-coded proteins promote the attachment of tail fibers to the phage baseplate, but neither is entirely essential.

P63

P63 is a nonstructural phage-coded protein that appears to catalyze fiber attachment. When P63 is added to reaction mixtures containing purified fibers and fiberless particles, the initial rate of fiber attachment is increased proportionally to the level of P63 present, but the final yield of infectious phage is limited by the level of either fibers or fiberless particles, suggesting that P63 acts catalytically (11).

P63 has been purified to near homogeneity, and the molecular weight of the active protein has been estimated at about 80,000 by gel filtration. Electrophoresis on SDS-polyacrylamide gels gives a subunit molecular weight of approximately 45,000, suggesting that the active form is a dimer. The reaction catalyzed by P63 shows no cofactor requirement and does not proceed detectably at 0°C (11). P63 also promotes the slow attachment of free A half fibers to fiberless particles, suggesting that its normal function is to facilitate interaction between the baseplate and the proximal end of the tail fiber (1, and unpublished observations).

Recently, we have found that a second protein, the wac (whisker antigen control) gene product, also promotes tail fiber attachment. Similar observations have been made independently by E. Terzagi, B. Terzagi, and D. Coombs (personal communication). The wac gene controls the antigenic determinants and presumably the structure of the T4 whiskers, slender filaments of about 20 by 400 Å that extend outward from the collar region at the base of the head (12, 13). Evidence that whiskers are involved in tail fiber attachment is presented in Fig. 2a. Purified whiskerless (wac^-) fiberless particles were

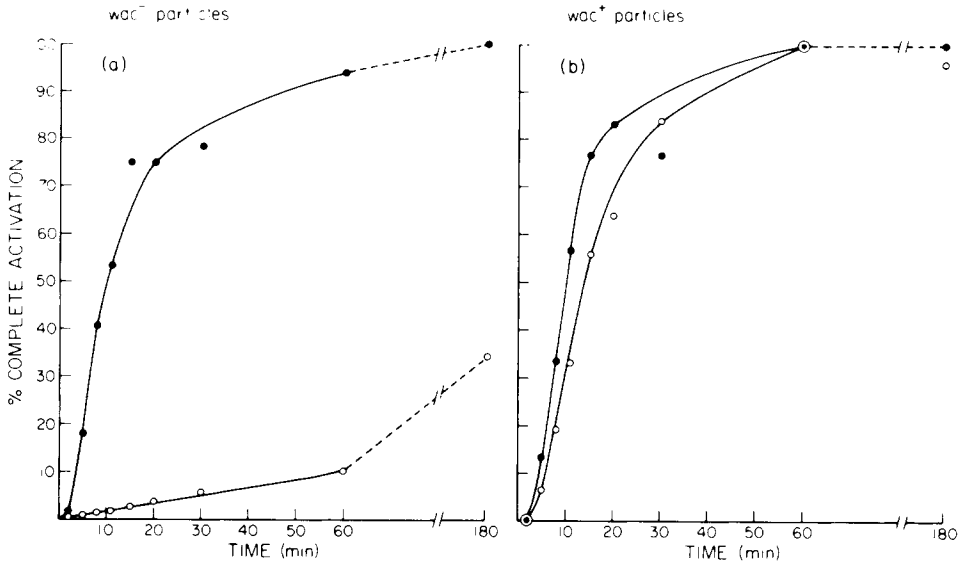

Fig. 2. Dependence of tail fiber attachment on Pwac. Tail fiber attachment was followed by the appearance of infectious virus in mixtures of purified fiberless particles and crude extracts containing tail fibers as previously described (10). Solid circles indicate results obtained with an extract containing both tail fibers and whisker antigen, made using a multiple amber mutant defective in genes 18, 23, 27, rIIA, and rIIB. Open circles indicate results obtained with an extract containing tail fibers but no whisker antigen, made using a multiple mutant defective in genes 18, 23, 27, rIIA, rIIB, and wac. (a) Extracts were incubated with whiskerless (wac^-) fiberless particles, purified from a defective lysate made using a multiple amber mutant defective in genes 34, 36, 37, 38, and wac. (b) Extracts were incubated with fiberless particles carrying whiskers (wac^+), purified from a defective lysate made using a multiple amber mutant defective in genes 34, 36, 37, and 38. Details of these experiments will be published elsewhere.

incubated with crude extracts containing either tail fibers plus whisker antigen (solid circles) or tail fibers minus whisker antigen (open circles). The rate of tail fiber attachment as measured by activation of the particles to infectious phage is strongly dependent upon the presence of whisker antigen in the extract. In the control experiment (Fig. 2b), particles that have whiskers (wac^+) are activated at essentially the same rate whether or not whisker antigen is present in the extract. The mechanism by which whiskers promote fiber attachment is under investigation.

DISCUSSION

Assembly and attachment of bacteriophage T4 tail fibers are clearly not simple self-assembly processes. Of the eight phage gene products required for efficient assembly and attachment, four are not structural components of the completed tail fiber. These four proteins are particularly interesting in that they appear to promote the formation

of noncovalent bonds between structural subunits. Two of the four proteins (P38 and P57) are required for the association of the major structural subunits of the distal half fiber and the other two (P63 and Pwac) facilitate the attachment of the completed fiber to the phage particle. The mechanisms of these reactions are not yet understood. However, our findings suggest that accessory proteins promoting the assembly of supramolecular structures may be a general phenomenon, and that such proteins should be looked for in other systems.

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