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Bacteriophage T4 whiskers: A rudimentary environment-sensing device

(virus infection/virus–host interaction)

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ABSTRACT **The 400 Å filaments or "whiskers," which extend outward from the collar region of the phage, control retraction and extension of the tail fibers in response to certain environmental conditions. The tail fibers of normal phage retract in the absence of a required adsorption cofactor, at low pH, at low ionic strength, at low temperature, and at high concentrations of polyethylene glycol. The tail fibers of mutant whiskerless (*wac*) phage still retract under the first two conditions, but not the last three. Antibodies to whiskers neutralize T4, probably by fixing tail fibers in the retracted configuration. Phage with retracted tail fibers adsorb poorly to host bacterial cells, and their adsorption rate increases as the fibers become extended. These results suggest that one function of the whiskers is to retract the tail fibers and thereby prevent adsorption to host cells under certain conditions that might be unfavorable for production of phage progeny following infection.**

The whiskers of bacteriophage T4 are slender filaments with dimensions of about 20 by 400 Å, which extend outward from the collar region at the base of the phage head. Although clearly visible in some early electron micrographs (for example, see ref. 1), they have been recognized only recently as distinct phage organelles (2, 3), and their function is not yet fully understood (4). The presence of whiskers depends on the expression of a gene designated *wac* [whisker antigen control (4)], which lies between genes 12 and 13 on the T4 genetic map (5). The whiskers are nonessential for phage growth, since amber (*am*) mutants defective in the *wac* gene, under nonpermissive conditions, form plaques somewhat smaller than those of wild-type T4 at normal plating efficiency (5). Such mutant phage are referred to throughout this paper as *wac*, while phage that carry the normal allele and have whiskers are designated *wac*$^+$.

Several *wac* mutants were selected initially as phage that would adsorb to host cells in the presence of polyethylene glycol (PEG) concentrations that inhibit adsorption of wild-type T4, presumably by causing its tail fibers to assume the retracted (P_R) configuration along the sheath instead of the extended (P_E) configuration (5). It has been known for many years that phage in the P_R state have a sedimentation constant of about 1000 S and adsorb poorly to bacteria, while phage in the P_E state have a sedimentation constant of 700 S–800 S and can adsorb efficiently to bacteria (see refs. 6 and 7, and earlier references in these papers). Phage in buffered saline solutions assume the P_R state at low pH ($\leq$5) and, for cofactor-requiring strains, in the absence of tryptophan. They return to the P_E state at pH $\geq$6 and, for cofactor-requiring strains, in the presence of tryptophan (6). More recent observations (8), extended in this paper, show that there is an ionic strength effect as well; phage assume the 1000 S, P_R state in 0.01 M phosphate buffer at pH 7 and return to the P_E state in the same buffer at higher salt concentrations.

The relative insensitivity of whiskerless phage to PEG suggested that whiskers might play the intriguing role of structural regulatory elements that prevent the phage from infecting host cells under certain conditions unfavorable for phage multiplication, by holding the tail fibers in the P_R configuration. To test this notion, we have compared *wac*$^+$ and *wac* phage with regard to the effects of several environmental variables on tail fiber retraction, as measured by sedimentation rate, sensitivity to neutralizing antibodies to whiskers, and rate of adsorption to host cells.

MATERIALS AND METHODS

Media and Reagents. H broth was used for liquid culture of bacteria and phage, and EHA top and bottom agar were used for plating (9). PEG (Carbowax 4000) was from Union Carbide Co., and papain from Calbiochem.

Phage Strains are all derivatives of T4D from the Caltech collection except as noted. The original *wac* mutant *am*E727J, obtained from a backcross of *am*E727 to wild type (4) and shown to carry a nonlethal *am* mutation in the *wac* gene, was given to us by Dr. F. Frankel and used for all experiments with *wac* phage. The PEG-resistant mutant CBW11B, shown to carry a nonlethal gene 36 mutation that impairs interaction between whiskers and tail fibers (5), was donated by R. Vanderslice. *wac*$^+$ rapid lysis mutants *r48* and *rdf41*, which give large *r* plaques, were often used in place of wild-type T4D. The gene defects in the following multiple *am* mutants are indicated in parentheses. X4E (34$^-$, 35$^-$, 37$^-$, 38$^-$) was used for preparation of tail-fiberless particles as described previously (10). X723 (34$^-$, 35$^-$, 37$^-$, 38$^-$, *wac*), obtained from a cross of *am*E727J with X4E, was used to prepare whiskerless tail-fiberless particles by the same procedure. X122 (23$^-$, 34$^-$, 37$^-$, 38$^-$) was used to prepare purified complete tails according to King (11).

***Escherichia coli* Bacterial Strains** were used as follows: CR63 (permissive for *am* mutants) for preparation of phage stocks and for genetic crosses; S/6 (nonpermissive) for preparation of *wac* phage and for plaque assay of mixed *wac* and *wac*$^+$ phage (*wac* phage produce small plaques on S/6 that are clearly distinguishable from wild-type or *r* plaques); and B/5 (nonpermissive) for preparation of defective lysates and for measurement of phage adsorption.

Methods not described were as reported previously (10).

RESULTS

Lack of Whiskers Affects the Sedimentation Behavior of Phage in Neutral Salt Solutions. At pH 7 in 0.01 M phosphate buffer containing 0.10 M NaCl, tail-fiberless, whiskerless, and normal (*r48*) phage particles are clearly separated (Fig. 1a). Assuming a sedimentation constant of 1000 S for

Abbreviations: PEG, polyethylene glycol; *am*, amber (suppressor-sensitive).

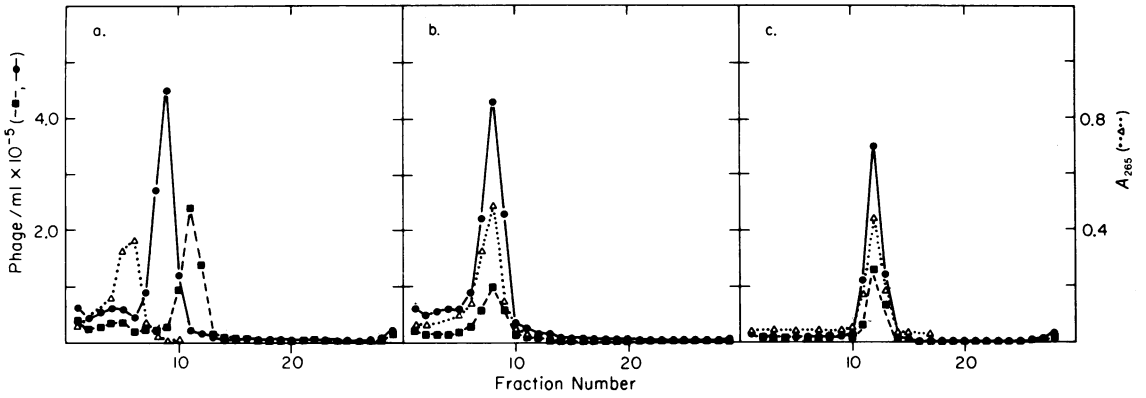

FIG. 1. Sedimentation patterns of normal, whiskerless, and tail-fiberless T4 particles under three conditions. Linear sucrose gradients (5–20%) of 11 ml on a 0.5 ml cushion of 50% sucrose contained 0.01 M sodium phosphate at the indicated pH, 0.001 M $MgSO_4$, 100 μg/ml of gelatin, and NaCl as indicated. Samples (0.05 ml) containing 8×10^7 *r48*, 6×10^7 *wac*, and 6×10^{11} (5 A_{265} units) tail-fiberless particles were layered onto the gradients and sedimented at 17,000 rpm (average 35,000 × *g*) for 50 min at 20° unless otherwise indicated. Fractions were diluted and assayed for infectivity by plating on S/6 bacteria. The large plaques formed by *r48* (*wac*$^+$) phage (●) were clearly distinguishable from the small plaques formed by *wac* phage (■). The tail-fiberless X4E particles used as a marker were assayed by absorbance at 265 nm (▲). Recoveries from gradients were ≥80% for A_{265}, 50–90% for *r48* phage, and 30–70% for *wac* phage. In general, recoveries decreased with decreasing salt concentration in the gradient. (a) pH 7, 0.10 M NaCl. (b) pH 5, 0.10 M NaCl. (c) pH 7, no NaCl; centrifugation was for 45 rather than 50 min.

fiberless particles (6), the sedimentation constants of *r48* and *wac* phage can be estimated from these profiles to be 860 S and 760 S, respectively. At pH 7 in 0.01 M phosphate buffer alone, or at pH 5 in 0.10 M NaCl, all three particles sediment together (Fig. 1b and c), indicating that under these conditions the presence or absence of either whiskers or fibers do not affect sedimentation. These results are as expected if at low salt concentration or low pH the fibers are bound up against the sheath and head in the retracted (P_R) configuration, whereas at 0.10 M salt and neutral pH the fibers of *r48* are partially extended, and those of *wac* are more completely extended. At higher salt concentrations, the sedimentation constant of *wac* particles decreases slightly, but never to less than 750 S.

We have assumed that these differences in sedimentation behavior are due only to changes in tail fiber configuration (see ref. 7 and *Discussion*). If it is further assumed that *wac* fibers are fully extended in the 750S condition, then a coefficient of fiber extension (C_E) for *r48* can be calculated from the sedimentation constants as

$$C_E = \frac{S_{fp} - S_{r48}}{S_{fp} - S_{wac}}$$

where S_{fp}, S_{r48}, and S_{wac} represent the sedimentation constants of tail-fiberless particles, *r48* phage, and *wac* phage particles, respectively. Under most conditions, this equation can be used in the more general form

$$C_E = \frac{1000\,S - S_x}{250\,S}$$

to calculate the degree of fiber extension C_E for any phage from its sedimentation constant S_x.*

As shown in Fig. 2 (solid symbols) the value of C_E increases with NaCl concentration at pH 7, and the responses of *wac* and *wac*$^+$ phage are quite different. Both phage appear to have tail fibers fully retracted in the absence of NaCl, but at 0.025 M NaCl the fibers of *wac* phage are almost fully extended whereas those of *wac*$^+$ phage are still almost completely retracted.

The finding that T4 *wac* mutants could adsorb to host cells at PEG concentrations that block wild-type T4 adsorption suggested that whiskers might mediate tail fiber retraction in the presence of PEG (5). It further suggested that whiskers also might mediate the tail fiber retraction observed by earlier workers (6) in response to low temperature, low pH, and, for certain cofactor-requiring strains of T4, absence of tryptophan. These suggestions were tested by comparing the sedimentation behavior of *wac* and *wac*$^+$ phage under various conditions, with the results shown in Table 1. These experiments confirm previous indications that the whiskers are required for tail fiber retraction at 5° (4) and enhance retraction in the presence of PEG (5). However, the results also indicate that whiskers are not required for retraction in response to low pH or absence of tryptophan.

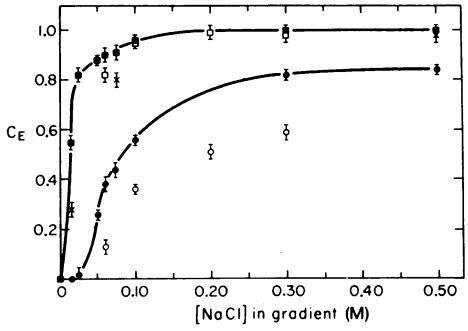

FIG. 2. Dependence of tail fiber extension on salt concentration and temperature. Coefficients of tail fiber extension (C_E) were calculated as described in the *text*, from sedimentation profiles obtained as in Fig. 1, except that measurements indicated by open symbols were made at 5° rather than 20°. Vertical bars indicate standard errors. Points with smaller error bars are average values from two or three independent determinations. (■), *wac* phage, 20°; (●), *r48* phage, 20°; (□), *wac* phage, 5°; (○), *r48* phage, 5°; (×), CBW11B phage, 20°.

* In 22 gradients run as described for Figs. 1a and b over a period of several months under a variety of ionic conditions, the mean number of fractions traveled by fiberless particles from the top of the gradient was 21.4 ± 1.4 (SD), with no apparent correlation to ionic strength or pH. Therefore, we have assumed in calculating C_E values that the sedimentation constant of fiberless particles is 1000 S under all of the conditions investigated, and that small apparent differences in their sedimentation (compare Fig. 1a and b) are due to uncontrolled variations in the sucrose gradients or in centrifuge temperature.

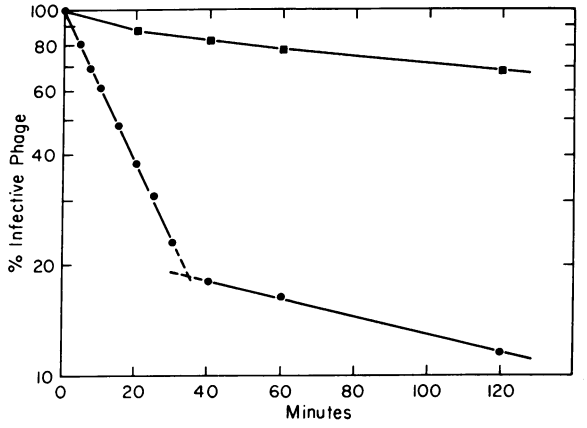

FIG. 3. Neutralization of *wac* and *wac*$^+$ phage by anti-whisker serum. Rabbit antiserum to tail-fiberless particles (11) was diluted into buffer I (0.01 M sodium phosphate, 0.005 M $MgSO_4$, pH 7) containing 1 mg/ml of bovine serum albumin to give k_n = 10 min^{-1} at 46° with wild-type T4D phage, and incubated overnight at 46° with sufficient purified whiskerless, tail-fiberless particles to block >90% of the neutralizing antibody directed against them, as determined in previous experiments. The adsorbed serum, referred to in the text as anti-whisker serum, was used for neutralization experiments without further treatment. *r48* (*wac*$^+$) phage (●) or *wac* phage (■) were incubated with an appropriate serum dilution in the above buffer at 46°. At the times indicated, samples were withdrawn and plated for surviving infectious phage on S/6 bacteria.

Table 1. Effect of the *wac* mutation on tail fiber extension under various conditions

Conditions in gradient				C_E ± 0.03	
Temp. (°C)	pH	[NaCl] (M)	Other	*wac*$^+$	*wac*
20	7	0.10		0.56	0.96
20	7	0.06		0.38	0.90
20	7	0.025		0.02	0.82
20	7	0.10	20 mg/ml of PEG	0.16	0.76
5	7	0.10		0.36	0.95
20	5	0.10		0.00	0.00
20	7	0.06	15 μg/ml of Trp (T4B)	0.52	0.88
20	7	0.06	no Trp (T4B)	0.04	0.04

C_E values were determined as in Figs. 1 and 2. T4B is genetically distinct from T4D and requires tryptophan (Trp) for tail fiber extension and adsorption to bacteria (17). Since the genetic determinant for this requirement maps in the tail fiber region, a tryptophan-requiring *wac* hybrid was obtained by crossing T4B with *am*X723 (*Materials and Methods*) and selecting progeny that plated on S/6 with the small-plaque phenotype characteristic of the *wac* defect. A stock made from such a plaque was shown to be insensitive to anti-whisker serum relative to the original T4B, and to require tryptophan for adsorption to bacteria.

Further experiments showed that low temperature decreased fiber extension in *wac*$^+$ phage at several salt concentrations, but affected *wac* phage only slightly at the lowest salt concentration tested (Fig. 2, open symbols).

In their study of PEG-resistant mutants, Follansbee *et al.* (5) found that in addition to the *wac* mutations, a mutation in gene 36 designated CBW11B also enhanced adsorption in the presence of PEG. This finding suggested that the protein coded by gene 36 (*p*36), a structural component of the distal half of the tail fiber adjacent to the central kink (2, 12), might be the site of whisker interaction with tail fibers. Consistent with this interpretation, sedimentation experiments with CBW11B showed that it is intermediate between *wac* and *wac*$^+$ phage in its retraction of tail fibers with decreasing salt concentration (Fig. 2, ×'s).

Anti-Whisker Antibodies Neutralize Phage with Retracted Tail Fibers. Most of the neutralizing activity of rabbit anti-T4 serum is directed against the phage tail fibers (13). Antiserum prepared against T4 tail-fiberless particles neutralizes phage at a slower rate, at least partly by combining with the phage baseplate (11, 13). However, when such antiparticle serum was adsorbed with purified T4 tails or tail-fiberless, whiskerless T4 particles, made using the appropriate *am* mutants, only about 70% of the neutralizing activity could be blocked. The residual activity could be blocked by lysates of nonpermissive cells infected with *am* mutants defective in any known late gene except *wac*, suggesting that this neutralizing activity is directed against the whiskers.

Anti-whisker serum gave a biphasic killing curve for *wac*$^+$ phage (Fig. 3). Neutralization to about 20% survival proceeded with first-order kinetics, and a rate constant (k_n) of about 3 min^{-1} for the undiluted serum. The remaining phage were neutralized at about a 7-fold lower rate (k_n = 0.4 min^{-1}), to <1% survival in some experiments. By contrast, the serum neutralized *wac* phage at a slow constant rate with k_n = 0.3 min^{-1}. If the slow neutralizing activity for both phages is assumed to be due to residual antibase-plate antibodies still present in the adsorbed serum, then the results with *wac*$^+$ phage suggest that only about 80% of these particles can be neutralized by reaction of the whiskers with antibodies under these conditions.

Several lines of evidence suggest that anti-whisker antibodies neutralize *wac*$^+$ phage by fixing tail fibers in the retracted state. First, the serum does not kill phage irreversibly, since *wac*$^+$ phage neutralized to <10% survival were restored to >90% of the original infective titer by treatment with 1.0 mg/ml of papain for 60 min at 37° (data not shown). Second, neutralized *wac*$^+$ phage were shown to adsorb very poorly to host cells compared to serum-treated *wac* phage (by incubation with bacteria, centrifugation, and assay of unadsorbed phage in the supernatant solution after reactivation with papain; data not shown). Third, the initial k_n for *wac*$^+$ phage is dependent on salt concentration. As shown in Table 2, k_n decreases by a factor of about 7 for *r48* as [NaCl] is increased, and shows an abrupt drop in the range of salt concentrations over which the fibers extend as measured by sedimentation (Fig. 2, solid circles). The salt dependence of k_n for CBW11B is quite different, but again the drop occurs in the range of increasing salt concentrations over which fibers extend (Fig. 2, ×'s). These results are not due to failure of antibodies to react with whiskers at high salt concentration. When *wac*$^+$ phage were treated with serum at high [NaCl] under conditions of slow inactivation (80% survival after 50 min) the survivors acquired resistance to PEG-inhibition of adsorption to bacteria, comparable to that of *wac* phage (data not shown). The simplest explanation for this effect is that the whiskers, by reacting with antibody when the fibers are extended, become unable to mediate subsequent tail fiber retraction. The biphasic nature of the neutralization curve in Fig. 3 also is consistent with this explanation, if the slowly neutralized fraction of phage represents particles on which, by chance, several whiskers reacted with antibody at times when the corresponding tail fibers were extended.

Rate of Phage Adsorption to Bacterial Cells Can Be Re-

Table 2. Salt dependence of initial neutralization rate for *r48* and CBW11B phage by anti-whisker serum

[NaCl] (M)	k_n (min^{-1})	
	r48	CBW11B
0.000	0.083	0.059
0.015	0.069	0.017
0.030	0.041	0.010
0.045	0.025	0.012
0.060	0.011	0.011
0.090	0.012	0.010

Values of k_n were calculated from neutralization kinetics determined as described in the legend to Fig. 3. The buffer contained a constant concentration of anti-whisker serum and various concentrations of NaCl as indicated.

lated to Degree of Tail Fiber Extension. The previously observed PEG-resistance and other properties of *wac* mutant phage (4, 5) suggested that whiskers might control adsorption rates by mediating tail fiber retraction, since phage in the P_R state adsorb poorly to bacterial host cells (6). To quantitate the relationship between adsorption and fiber extension, adsorption rate constants were determined at 20° for *wac*$^+$ and *wac* phage at several NaCl concentrations. The experiments were designed to measure the fraction of phage that remained unadsorbed after incubation with bacteria and rapid centrifugation to remove all adsorbed phage (Table 3). Since [NaCl] may directly affect the interaction of phage with the cell surface as well as affecting whisker–fiber interaction, the ratio of rate constants for *wac*$^+$ and *wac* phage is probably the best parameter for assessing the relationship of salt-dependent fiber extension to adsorption rate. As shown in Table 3, this ratio increases from about 0.4 to 1.0 as [NaCl] is increased from very low concentration to a level at which the tail fibers of *wac*$^+$ phage are about 25% extended as measured by sedimentation.

Table 3. Salt dependence of *wac*$^+$ and *wac* phage adsorption rates to bacterial cells

[NaCl] (M)	k_a (min^{-1})		k_a ratio *wac*$^+$/*wac*
	wac$^+$	*wac*	
0.000	0.15	0.37	0.39
0.015	0.33	0.52	0.64
0.025	0.36	0.47	0.76
0.050	0.51	0.50	1.01
0.060	0.54	0.53	1.02
0.100	0.50	0.47	1.05

B/5 cells were grown in H broth to early log phase, collected by centrifugation, drained, and resuspended in buffer II (0.01 M sodium phosphate, 0.001 M $MgSO_4$, pH 7) at 5×10^9 cells per ml. A 0.95 ml sample of *rdf41* (*wac*$^+$) or *wac* phage at 2.5×10^5 per ml in buffer II that contained NaCl as indicated was prewarmed to 20°, and at 0 min 2.5×10^8 cells were added and mixed rapidly. After a 5 min incubation at 20°, the suspension was chilled in an ice bath for about 1 min, centrifuged for 2 min at $3500 \times g$, and a sample of the supernatant was assayed for unadsorbed phage by plating on S/6 bacteria. The adsorption rate constant (k_a) was computed from the fraction of phage that remained unadsorbed (f) assuming first order kinetics, that is, $\ln f = -k_a t$, where t = time in min. Thus a rate constant of 0.54 min^{-1} is equivalent to 6.6% unadsorbed phage at 5 min. Under these conditions, disappearance of unadsorbed phage from the suspension was approximately first order down to an unadsorbed fraction of <5%.

DISCUSSION

Our interpretation of the sedimentation experiments is based upon two assumptions: that the observed variations in sedimentation rate with centrifugation conditions are due entirely to changes in tail fiber configuration, rather than other changes in particle shape, and that the differences in sedimentation between *wac* and *wac*$^+$ phage are due entirely to whisker-mediated effects on tail fiber configuration. The first assumption has been justified convincingly by Gordon (7), and is consistent with our failure to observe any reproducible variation in tail-fiberless particle sedimentation with ionic conditions.* The second assumption is supported by the following arguments. First, *wac* and *wac*$^+$ phage cosediment under conditions where the tail fibers are retracted. Second, the functional test of adsorption to bacteria indicates that there are differences in tail fiber configuration between *wac* and *wac*$^+$ phage in the low range of 0.01–0.05 M NaCl. Third, the tail fiber mutation CBW11B causes *wac*$^+$ phage to sediment similarly to *wac* phage; specifically, at 0.50 M NaCl, pH 7, where *wac* and *wac*$^+$ phage differ in sedimentation properties (Fig. 2), *wac* and CBW11B phage show the same sedimentation constant within experimental error (752 ± 7 S and 756 ± 7 S, respectively).

If the phage particle could exist only in either of two interconvertible states, P_R and P_E, then the parameter C_E would be a measure of the equilibrium position of the interconversion reaction $P_R \rightleftarrows P_E$. Therefore, C_E would represent the fraction of the population that is in the P_E state at any instant, or alternatively, the fraction of time on the average that each particle spends in the P_E state, for example during a sedimentation experiment. In practice, a simple quantitative correlation of C_E with rates of neutralization by serum and adsorption to bacteria is not necessarily expected, because (*a*) intermediate states probably exist in which some of the fibers on individual particles are retracted and others extended, and (*b*) less than six fibers in the extended configuration are probably sufficient for successful adsorption and injection (10). However, there is a clear qualitative correlation between C_E, neutralization, and adsorption.

Anti-whisker antibodies appear to neutralize *wac*$^+$ phage by immobilizing the tail fibers in the P_R configuration, and it seems most likely that the dependence of neutralization rate on [NaCl] reflects the dependence of tail fiber extension on [NaCl]. The adsorption of T4 to bacteria is at least a 2-step process that involves a reversible interaction of tail fibers with the cell surface, followed by irreversible attachment of the baseplate and injection of DNA (14–16). Disappearance of unadsorbed phage follows pseudo-first-order kinetics, because the concentration of receptors on the bacteria does not change appreciably during an adsorption experiment. Since reversible adsorption is the rate-limiting parameter measured in the experiment of Table 3, the results indicate that tail fiber configuration affects the initial tail-fiber-mediated step in the adsorption process. The rate of this step increases with increasing tail fiber extension, and appears to reach a plateau value of maximum efficiency when the fibers are about 25% extended as determined by sedimentation.

The experiments reported here extend a large number of observations in the classical as well as the more recent phage literature that tail fibers are retracted and adsorption inhibited under certain environmental conditions. Our results also help to explain the classically observed failure of T-even phages to infect host cells in low ionic strength media. The phage whiskers clearly mediate tail fiber retraction in re-

sponse to decreases in ionic strength and temperature. However, they are not required for retraction at very low ionic strength, at low pH, or in the absence of the adsorption cofactor tryptophan for a tryptophan-requiring strain. These findings imply that there is at least one other set of sites on the phage particle besides the whiskers that can maintain tail fibers in the P_R configuration under certain conditions. Both sheath (17) and baseplate (18) have been suggested for the sites that respond to adsorption cofactors, but conclusive evidence for either is lacking.

The mechanism of whisker-mediated tail fiber retraction probably involves association between the whiskers and $p36$ (5), a protein in the distal half of the tail fiber near the central kink (2, 12). When a tail fiber is lying along the tail sheath in the P_R configuration, the central kink is about 300 Å from the collar, within easy reach of a 400-Å whisker. Since the equilibrium of the retraction–extension reaction is shifted toward retraction by decreases in either ionic strength or temperature, the whisker–fiber association must involve interaction of polar rather than nonpolar groups on the two proteins.

Given the role of the whiskers in mediating the responses to temperature and ionic strength, it is tempting to speculate on the functional significance of these organelles. Tail fiber retraction may decrease the probability of accidentally triggering the baseplate conformational changes that lead to sheath contraction (19). In addition, the whiskers are required for efficient attachment of tail fibers during viral assembly. Evidence for this function is presented elsewhere (ref 20; E. Terzaghi, B. Terzaghi, and D. Coombs, referred to in ref. 21; W. B. Wood and M. P. Conley, submitted for publication). Another function of the whiskers is apparently to control the adsorption behavior of the virus. Bacteriophage must be adapted to survival in diverse environments, including animal intestines, where the bacterial concentration is high and conditions are suitable for phage multiplication, as well as sewers and natural aquatic environments where bacteria are relatively scarce and the temperature and ionic concentrations may be too low to permit phage multiplication. Under the latter conditions, it should be advantageous to the phage to refrain from adsorption and injection. The whiskers allow T4 to "sense" the environment, retract its tail fibers, and thereby prevent adsorption under certain conditions. On the one hand, tail fiber retraction is simply a conformational change, caused by an ionic strength- and temperature-dependent interaction between viral protein subunits. On the other hand, it can be viewed as a rudimentary behavioral response that probably has survival value for the virus.

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