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A REGULATOR PROTEIN FOR THE LENGTH DETERMINATION OF BACTERIOPHAGE LAMBDA TAIL*

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Mutants in gene U of phage λ produce polytails. Those polytails have a tail fiber and a basal part like normal tails, but their major tubular part is longer than that of normal tails and shows a wide length distribution.

We established the morphogenetic pathway of the λ tail and found that U gene product (pU) acts throughout the assembly of the major tail protein (pV). Polytails in U^- lysate are activated by pU in vitro and form long-tailed phage which are infectious to a small extent.

If the formation of the basal part of the tail is blocked, pV (the major tail protein) remains unassembled as long as pU is present in the cell. However, we found that part of pV assembles into giant polytubes of several microns in length in lysates of a double mutant $U^- \cdot H^-$ in which both the basal part and pU are absent.

pV in purified tails can be dissociated into disks (about 10S) or smaller units (about 2.5S) in vitro under extreme conditions. The disks form polytubes efficiently under physiological conditions, but the smaller units do not form polytubes efficiently. The smaller units have in vitro complementation activity with V^- lysate. In vitro complementation activities with V^-, U^-, and Z^- lysates are detected in the dialyzed extracts of SDS gel electrophoresis of purified tails. The molecular weights of the polypeptide chains containing those activities are estimated to be 31,000, 14,000 and 20,000 daltons, respectively.

INTRODUCTION

The tail of bacteriophage λ has a relatively simple structure: a 1500 Å tube is ended by a small basal part and a tail fiber (1, Eiserling quoted in 2). At least 11 genes (genes Z, U, V, G, T, H, M, L, K, I, J) control the morphogenesis of the λ tail (3) and 7 or 8 of the 11

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Nos. I–III are Kühl and Katsura (Ref. 9) and Katsura and Kühl (Refs. 14, 11).

gene products have been identified by SDS polyacrylamide gel electrophoresis (4). Among these, the product of gene V is the major tail protein (5). The product of gene J is most probably the tail fiber, because among tail genes only gene J is responsible for the production of serum blocking power (6, 7), and because host range mutants map in gene J (8).

No tail-related structure has been found by electron microscopy in defective lysates of tail mutants except the following two cases: Z^- mutants produce phage-like particles and defective tails (S. Casjens and R. Hendrix, personal communication; K. Lickfeld and B. Hohn, personal communication; 9) and polytails are found in U^- lysates (8, 10). Polytails in U^- lysates have a basal part and a tail fiber like normal tails, but the major tubular part is longer than that of normal tails and shows a wide length distribution, whereas the length of a normal tail is exactly determined. The number of pU molecules exceeds the number of pV molecules in the cell, but only a very small amount of pU, if any, is incorporated into phage particles (4).

Thus it seems that pU regulates the assembly of the major tail protein (pV) and determines the normal length of the tail either directly or indirectly. In this report we describe some properties of pU and pV and discuss possible mechanisms of the regulatory function of pU.

MATERIALS AND METHODS

Bacterial and Phage Strains

Lysogens of the type 594 [λcI857 $tail^-$ (or $head^-$) Sam7] were used for preparing defective lysates. The tail mutations are Zam405, Uam413, Vam750, Gam901, Ham866, Mam888, Lam756, Kam768, Iam838, and Jam27: the head mutations are Aam32 and Eam4. Ymel $suIII^+$ was used as indicator bacterium.

Media

M9 supplemented with 1% casamino acids was used for bacterial culture. LA plates and Tryptone top agar were used for plating, λ dilution buffer (containing Tris) was used for phage dilution. Details are in Ref. 11.

Preparation of "Pregnant" Bacteria

Lysogens mentioned above were cultured at 32°C up to the bacterial titer of 3×10^8/ml, induced at 45°C for 8 min, and cultured further at 37°C for 1 to 3 hr. Then the "pregnant" bacteria were pelleted by low speed centrifugation.

Sucrose Gradient Centrifugation of Defective Lysates

Samples for sucrose gradient centrifugation were made by resuspension of pregnant bacteria in 1/200 volume of 0.2 M Tris-HCl (pH 8.8) with 40 μg/ml of DNase, lysis by freeze-thawing, and removal of debris by centrifugation.

0.1 ml of the sample with a marker (catalase: 11.3S) was layered on 3.5 ml of a 10 to 25% sucrose gradient in 0.2 M Tris (pH 7.8) and centrifuged in an SB405 rotor in

an IEC B-60 centrifuge at 58,000 rpm for 3.5 hr at 4°C.

In Vitro Complementation

Pregnant bacteria were resuspended in 1/400 to 1/200 volume of λ dilution buffer (for complementation with fractions of sucrose gradients) or 0.1 M Tris-HCl-20 mM $MgSO_4$ (pH 7.8) (for other purposes). Aliquots of the bacterial suspension were mixed with samples to be tested, lysed by freeze-thawing, incubated overnight at room temperature, and plated to assay phage titer.

Purification of Tails

Tails used for simple pH dissociation experiments were purified from E^- lysate or from A^- lysate essentially according to Bleviss and Easterbrook (12). Tails used for sucrose gradient centrifugation after dissociation by pH treatment and for dissociation with SDS or guanidine hydrochloride were further purified by sucrose gradient centrifugation.

Partial Purification of pU

pU was partially purified from V^- lysate by centrifugation, ammonium sulfate precipitation, and DEAE Sephadex A-25 column chromatography. After these purification steps, about 80% of the protein consisted of pU. Details of the purification procedure will be published elsewhere.

SDS Polyacrylamide Gel Electrophoresis

SDS gel electrophoresis was done according to Laemmli (13) except that the final concentration of acrylamide was 12.5% and a slab gel apparatus was used. Dissociated proteins from purified phage and from purified tails were run in one part of the gel and this part was stained with Coomassie Blue. About 0.5 mg of purified tails heated in the sample buffer was run in the other part of the same gel. This part of the gel (height × width × thickness = 90 mm × 25 mm × 1 mm) was used for the detection of in vitro complementation activities. It was cut horizontally into 23 fractions of the same height, and each fraction was further chopped into small cubes. 0.2 ml of the electrode buffer was added to each fraction and proteins were extracted from the gel by shaking overnight in the cold. Then the extracts were dialyzed twice (each for 2 hr) against 50 volumes of 8 M urea – 0.1 M Tris-HCl (pH 7.8) at room temperature for removal of SDS, and then twice (each for 2 hr) against 100 volumes of 0.1 M Tris-HCl – 20 mM $MgSO_4$ (pH 7.8) in the cold. The dialyzed extracts were tested for in vitro complementation activities according to the method mentioned above.

Dissociation of Tails by pH Treatment

Partially purified tails (0.3 mg/ml) in 0.02 M phosphate buffer (pH 7.0) were dialyzed against 100 volumes of buffer solutions of designated pH overnight at room temperature. Buffer solutions used were: Sørensen's glycine I buffer (glycine-NaCl-HCl)

for pH 2 to 3.5, 0.1 M acetate buffer for pH 4 and 5, 0.02 M histidine buffer for pH 6, 0.02 M phosphate buffer for pH 7, 0.1 M Tris-HCl buffer for pH 8 and 9, Sørensen's glycine II buffer (glycine-NaCl-NaOH) for pH 10 to 12.5 and 0.1 M NaOH for pH 13 (the exact pH was 12.85). Aliquots of the dialyzed samples were directly observed in the electron microscope. The rest was dialyzed against 0.02 M phosphate buffer (pH 7.0) and checked for polytube formation (by electron microscope) and residual tail titer. Tail titer was measured by plating after incubation with a J^- lysate containing excess heads for several hours.

Electron Microscopy

A drop of sample was applied to a grid with a carbon-coated collodion film, washed with a drop of distilled water, negatively stained with 1% uranyl acetate and observed in a Philips EM300 electron microscope.

Terminology

X^+ activity is the activity which complements X^- lysates in vitro, where X represents any tail gene. $(UH)^+$ activity is the activity which complements a $U^- \cdot H^-$ lysate.

$(H + V^-2S\ fr)^+$ activity, $(UH + pU)^+$ activity, and $(AZ + ZH)^+$ activity are the activities which complement the mixture of H^- lysate with the 2S fraction of V^- lysate, the mixture of $U^- \cdot H^-$ lysate with partially purified pU, and the mixture of $A^- \cdot Z^-$ lysate with a $Z^- \cdot H^-$ lysate, respectively.

RESULTS

Morphogenetic Pathway of the λ Tail

The morphogenetic pathway of the λ tail is shown in Fig. 1 (11). Of all tail genes only the position of gene T is unknown in the morphogenetic pathway because no amber mutant in this gene is available. Since the product of gene J is the tail fiber, λ tail assembly seems to progress from the distal part to the proximal part. A precursor which requires genes J, I, K, L, G, H, M for its formation [$(25S)_{II}$ in Fig. 1] serves as an initiator or a nucleator for the assembly of the major tail protein (pV). If the formation of this precursor is blocked by a mutation, pV remains unassembled as long as pU is present. No assembled form of pV is detected by electron microscopy and V^+ activity sediments usually at about 2.5S in these defective lysates. Both pU and pV act at the step between the $(25S)_{II}$ precursor and the Z^- tail in the morphogenetic pathway. Attempts to sequence the actions of pV and pU within this step were unsuccessful as shown below, and it seems that pU is required throughout the assembly of pV onto the 25S precursor.

We found an additional abnormal pathway in vitro (Fig. 1) (11, 14). In U^- lysates some polytails have an empty head, but most polytails have no head on them. These free polytails in U^- lysate are activated by pU, bind a head, and form long-tailed phage. The infectivity of this long-tailed phage, expressed as the number of plaque forming units per UV absorbance at 260 mμ is 0.04% of that of wild-type phage. Therefore, it seems that pU probably acts on the top of the polytails and prepares them for the attachment of a head. Without this action of pU the attachment of a head to the polytails is inefficient.

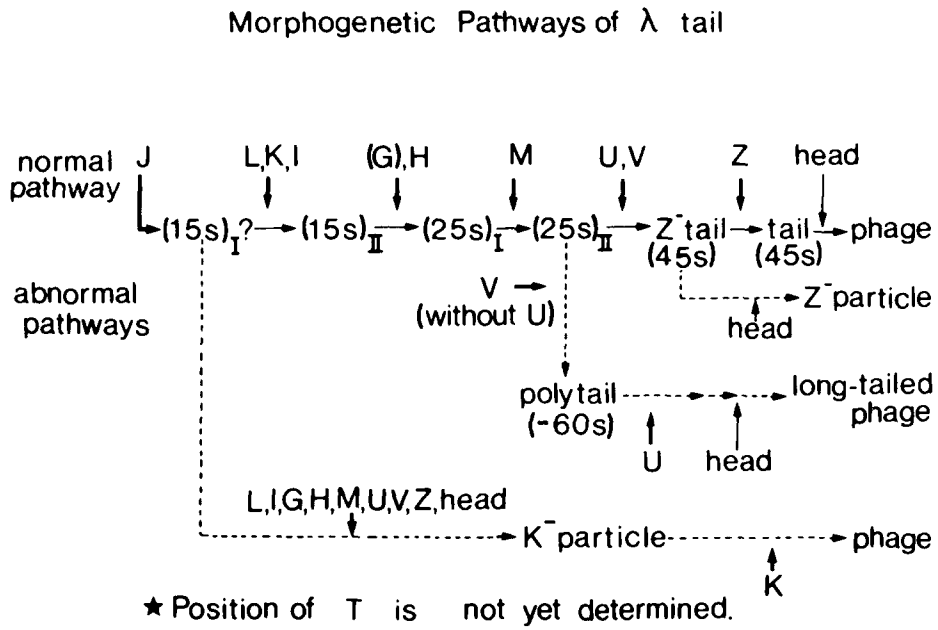


Fig. 1. Morphogenetic pathway of λ tail (11). The pathway via K^- particle is very minor and is not discussed in this paper.

Properties of a Double Mutant $U^- \cdot H^-$

Phenotype observed in the electron microscope. pV remains unassembled in H^- lysate in which the formation of both 25S precursors is blocked. However, we found giant polytubes (Fig. 2) in a $U^- \cdot H^-$ lysate in which both pU and the 25S precursors are absent. These giant polytubes have the same characteristic stacked disk structure as the tubular part of normal tails. But they are extremely long (several microns), and – unlike U^- polytails or normal tails – they have neither the basal part nor the tail fiber at either end. By complementing the $U^- \cdot H^-$ lysate with pU in vitro, we can bind a head at one end of the giant polytubes, although no increase of phage titer is detected. We conclude that pU keeps pV soluble and ready to interact with the 25S initiator of tail assembly. In the absence of both pU and the initiator some pV polymerizes into aberrant polytubes.

In vitro complementation. $U^- \cdot H^-$ does not complement either U^- or H^-, but it complements V^- in vitro. (Phage titer: $U^- \cdot H^- \sim 10^3$/ml; $H^- \sim 10^5$/ml; $V^- \sim 10^5$/ml; $U^- \cdot H^- + V^- \sim 10^9$/ml; $H^- + V^- \sim 10^{10}$/ml; each in 150-fold concentrated lysate.) Phage made by this in vitro complementation has a normal sedimentation coefficient and seems to be normal phage.

V^+ activity in $U^- \cdot H^-$ lysate sediments at 2.5S in a sucrose gradient. Therefore, we conclude that some of pV in $U^- \cdot H^-$ lysate remains unassembled, while the rest of pV forms giant polytubes.

pU is necessary throughout the normal assembly of pV. We can make only long-tailed phage and not normal phage in vitro from U^- polytails (11, 14). This means that pU acts in the normal pathway before the assembly of pV into polytails is completed. But we

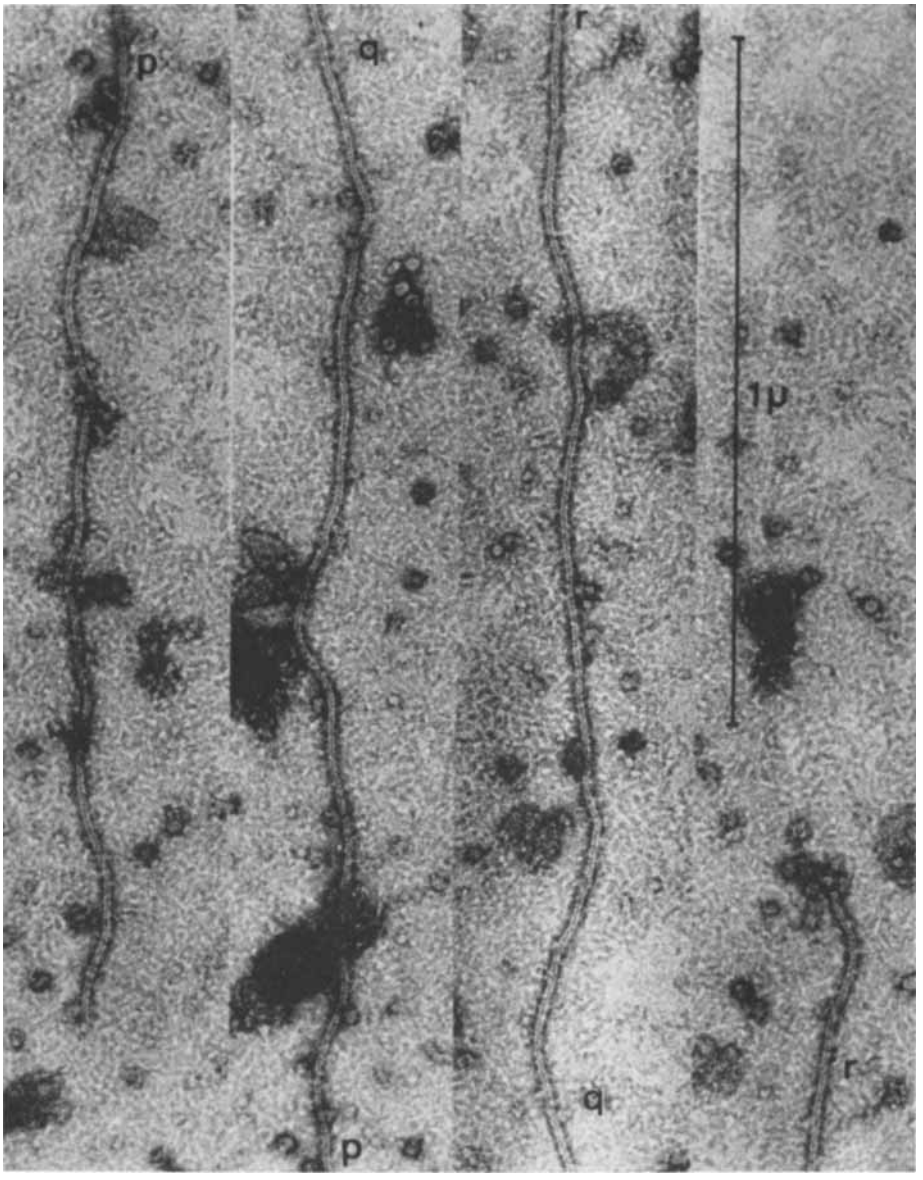
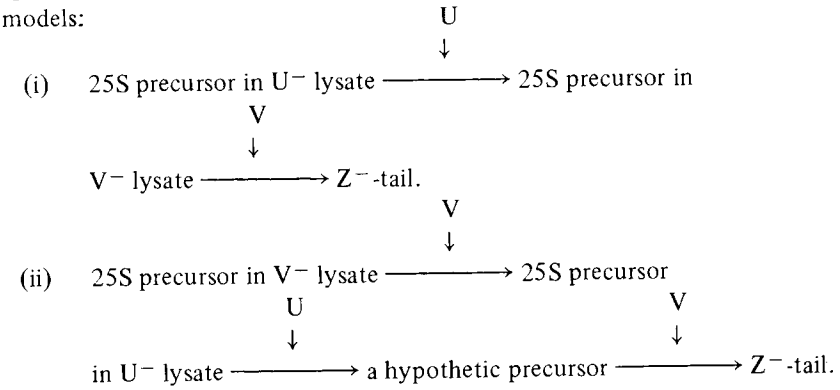


Fig. 2. Electron micrographs of a giant polytube in $U^{-} \cdot H^{-}$ lysate. This polytube is one of the shortest in $U^{-} \cdot H^{-}$ lysate. However, it is still so long that four micrographs were necessary to show the whole image with fine structure. The letters on the micrographs show the corresponding positions of the polytube.

have to decide whether pU is necessary throughout the assembly of pV or at a given step during or before the assembly of pV. For instance, one could assume the following two models:



The first model can be checked by whether the 25S precursor isolated from a V^- lysate has $(UH)^+$ activity or not, because $U^- \cdot H^-$ lysate contributes unassembled pV but not pU. The result is shown in Fig. 3. The 25S precursor from V^- does not complement $U^- \cdot H^-$ lysate in vitro. By the following control experiment we demonstrate that the 25S precursor has not lost its activity during isolation in the sucrose gradient. We mixed a $U^- \cdot H^-$ lysate with the 2S fraction of a V^- lysate in which pU is present. This mixture was added to each fraction of the sucrose gradient of V^- lysate. Then we found a peak of phage titer at 25S (Fig. 3). Therefore, we conclude that the first model is not true and pU does not react with the 25S precursor in U^- lysates to give an active nucleator for pV polymerization.

In order to test the second model a U^- lysate was mixed with partially purified pU (see Materials and Methods), incubated and centrifuged in a sucrose gradient. As shown in Fig. 4, the 25S fraction had no $(UH)^+$ activity no matter whether pU had been added to the U^- lysate before sucrose gradient centrifugation or not. Along the profile we could detect no $(UH)^+$ activity which we could attribute to the hypothetic precursor. The activity which is found at the bottom in the sucrose gradient in Fig. 4 is due to polytails activated by pU and not due to the hypothetic precursor in the normal pathway, because of the following reasons: 1) the peak of this activity sediments at 60S; 2) V^+ activity overlaps with this activity; and 3) phage made in this fraction sediments slowly like long-tailed phage. The 25S fraction of U^- lysates, with or without added pU, complements the mixture of $U^- \cdot H^-$ lysate with partially purified pU, which shows that at least part of the 25S precursor in U^- lysate remains active after incubation with pU and sucrose gradient centrifugation. Thus the second model is also disproved; it is ruled out that pU acts only at a given step during the polymerization of pV.

We conclude that these two experiments strongly suggest that pU is necessary throughout the assembly of pV.

Properties of Dissociated Tails

Dissociation of tails. Partially purified tails (about 0.3 mg/ml) were dissociated by pH treatment overnight and observed in the electron microscope. Aliquots of pH-treated tails were dialyzed against 20 mM phosphate buffer (pH 7.0) and checked for polytube formation (12) by electron microscopy. The results are summarized in Table I.

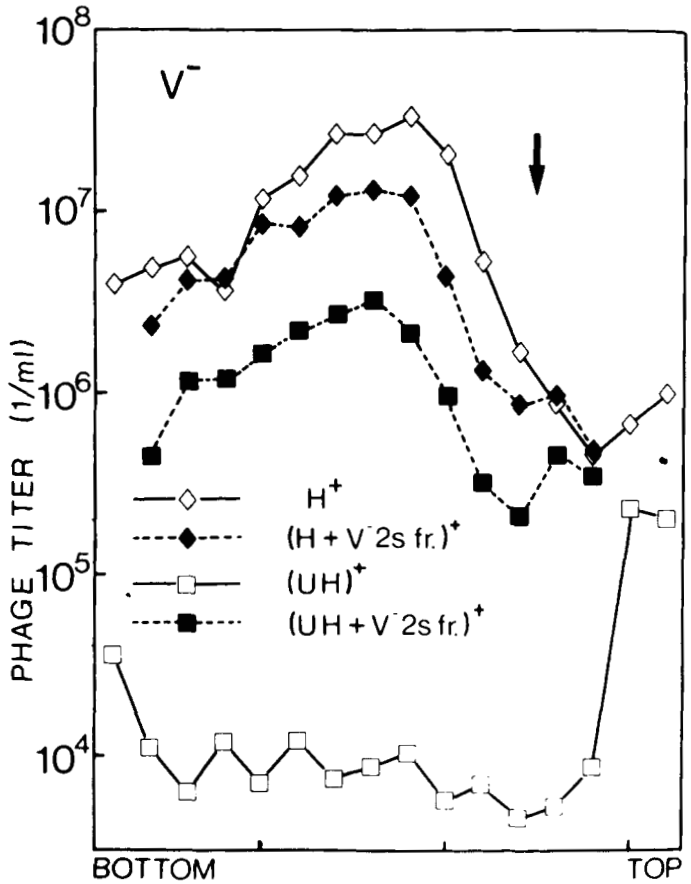


Fig. 3. Lack of $(UH)^+$ activity in the 25S precursor in V^- lysate. Sucrose gradient centrifugation and in vitro complementation were done according to Materials and Methods section. The arrow indicates the position of catalase (11.3S). The solid lines with open squares show $(UH)^+$ activity. The other three types of lines show that the 25S precursor is not denatured.

As reported by Bleviss and Easterbrook (12) tails disappear completely at pH 2 and ringlike structures appear instead (Fig. 6). After readjusting the pH to 7, large amounts of polytubes are formed from these pH 2 dissociated tails. In order to study this phenomenon in more detail, the product of pH 2 dissociated tails was centrifuged in a sucrose gradient at the same pH. As shown in Fig. 5, acid dissociated tails showed two peaks at 10S and at 2.5S. SDS gel electrophoresis showed that both peaks consisted mainly of pV. We found large numbers of rings by electron microscopy in the 10S fraction and no detectable structure in the 2.5S fraction. After readjusting the pH to 7 polytube formation was very abundant in the 10S fraction and very poor in the 2.5S fraction.

In contrast to acid dissociation, no rings are formed at alkaline pH as reported by Bleviss and Easterbrook (12). When we increase the pH stepwise, tails gradually disappear and dissociate into the 2.5S units. Polytube formation was observed almost only when part of the tails were dissociated and it was less efficient than the polytube formation after acid dissociation. At a pH of about 13, pV of the tails dissociated completely into the 2.5S units (Fig. 5). No detectable structure was observed by electron microscopy after we readjusted the pH to 7.

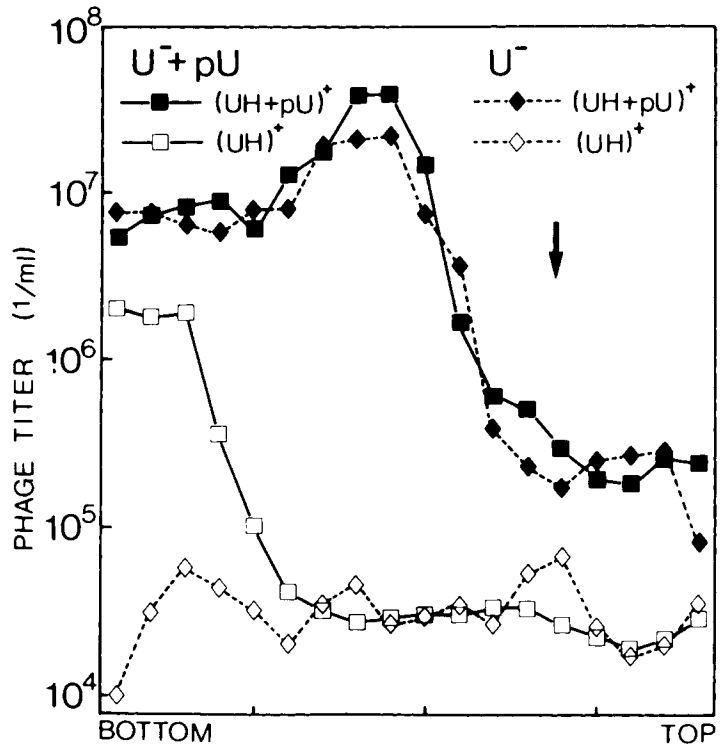


Fig. 4. $(UH)^+$ activity and $(UH + pU)^+$ activity in U^- lysate with and without the addition of pU. One-twentieth volume of partially purified pU (about 2 mg/ml) was added to the U^- lysate before sucrose gradient centrifugation. U^- lysate without the addition of pU was also centrifuged in a sucrose gradient. The results of two gradients are shown in one figure. Fractions of both of the two gradients were tested for vitro complementation activities with $U^- \cdot H^-$ lysate and with $U^- \cdot H^-$ lysate supplemented with one-twentieth volume of partially purified pU (about 2 mg/ml). The arrow indicates the position of catalase (11.3S).

TABLE I. Disruption of Tails by pH

pH	2.0	2.5	3.0	3.5	4.0	5.0	6.0 ~ 11.0	11.5	12.0	12.5	13.0
tail tube	–	–	+	+	+	+	+	+	+	–	–
tail fiber	–	–	±	+	+	+	+	±	–	–	–
ring	+	+	±	–	–	–	–	–	–	–	–
polytube	+	+	±	–	–	–	–	–	+	±	–
tail titer		< 0.02%			1%	20%	100%	1%		< 0.02%	

*Partially purified tails were treated with buffer solutions of various pH according to Materials and Methods section. Disappearance of the tail tube and the tail fiber and appearance of ringlike structures were observed by electron microscopy. Polytube formation and residual tail titer were checked after readjusting the pH to 7.

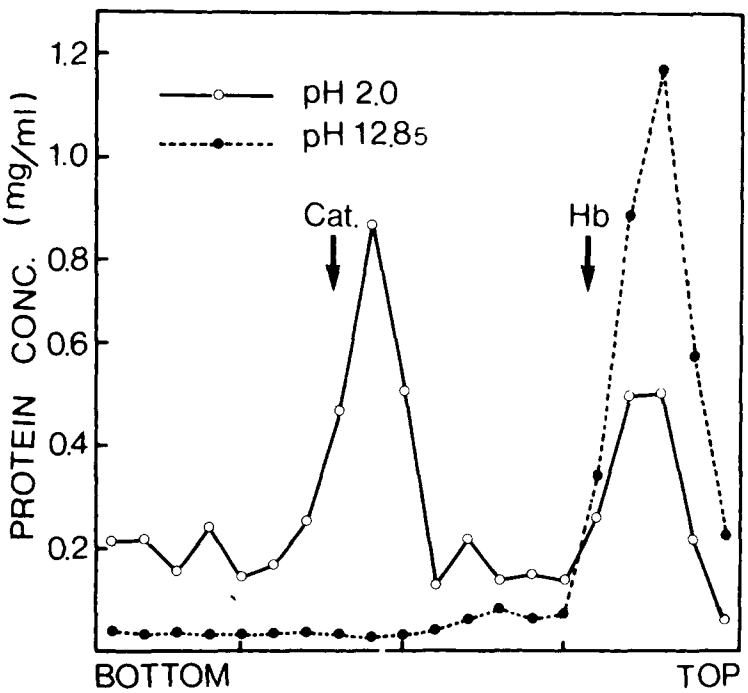


Fig. 5. Sucrose gradient centrifugation of dissociated tails. Purified tails (about 3 mg/ml) were dissociated at pH 2.0 and at pH 12.85, respectively, and centrifuged in 5–20% sucrose gradients in the same buffer solution as used for dissociation. (SB405 rotor and IEC B-60 centrifuge, 60,000 rpm, 4 hr at 15°C.) Mixture of catalase (11.3S) and hemoglobin (4.5S) was centrifuged in a 5–20% sucrose in 20 mM phosphate buffer (pH 7.0) in the same run of centrifugation, and their positions are indicated by arrows. Protein concentration of the fractions of the sucrose gradients was measured according to Lowry et al. (16).

Dissociation of tails by SDS or by guanidine hydrochloride showed essentially the same characteristics as alkaline dissociation.

In vitro complementation activities of tails dissociated with guanidine hydrochloride. Purified tails (about 1 mg/ml) were dialyzed against 6 M guanidine hydrochloride and then against 200 volumes of 0.1 M Tris-HCl – 20 mM $MgSO_4$ (pH 7.8) twice, each for 2 hr. By electron microscopy we observed nothing in this sample except some amorphous aggregates. This sample did not have G^+, L^+, K^+, I^+, and J^+ activities, but had Z^+, U^+, V^+, H^+, and M^+ activities. The increase of phage titer over background titer was about 3-fold, 10-fold, 10^4-fold, 3-fold, and 100-fold, respectively.

In vitro complementation activities extracted from SDS gel after electrophoresis of purified tails. Purified tails were dissociated by hot SDS and electrophoresed in SDS polyacrylamide gel according to Laemmli (13) using a slab gel apparatus. Part of the gel was stained, and the rest was cut into about 20 fractions. Proteins were extracted from each fraction, freed of SDS and complemented with Z^-, U^-, and V^-, lysates, respectively. The mixture of an $A^- \cdot Z^-$ lysate and a $Z^- \cdot H^-$ lysate was also used to detect Z^+ activity. [This method is more sensitive, because the effect of formation of aberrant Z^- particles

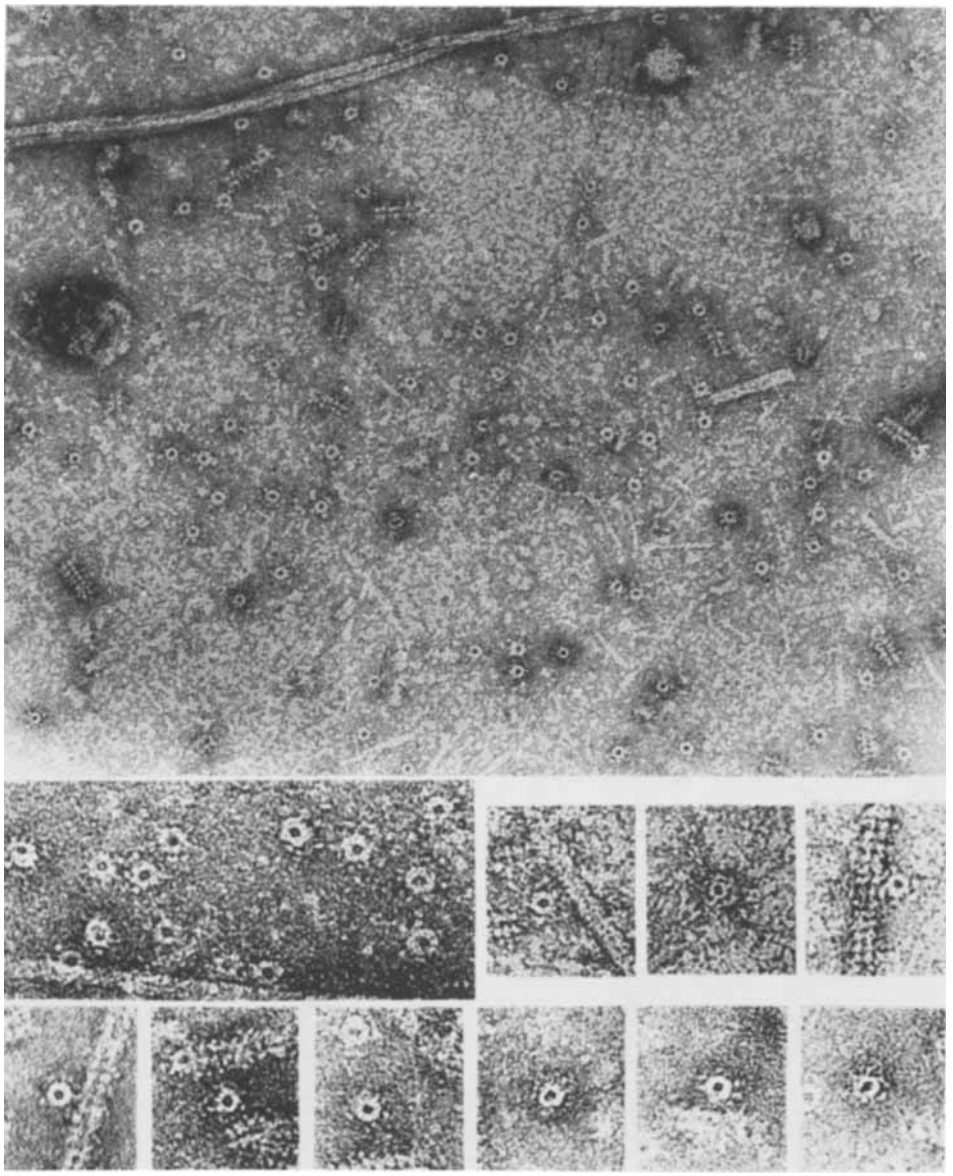


Fig. 6. Tails dissociated at pH 2.3. Many ringlike structures, tail fragments, and some bacterial pili (contamination) are seen in the micrograph. Magnification: × 202,500 and × 375,000 (inserted micrographs).

(11) is smaller if we use this mixture instead of a Z^- lysate.] As shown in Fig. 7, Z^+, U^+, and V^+ activities were found in the fractions corresponding to molecular weights of 20,000, 14,000, and 31,000 daltons, respectively. The molecular weights of the proteins carrying U^+ and V^+ activities agreed well with those of pU and pV, respectively (4). The band of pU was also detected by staining in SDS gel electrophoresis of both purified phage and tails. This band was at the same position as the major band of partially purified pU. pZ has not been identified in SDS gel electrophoresis, but the molecular weight of

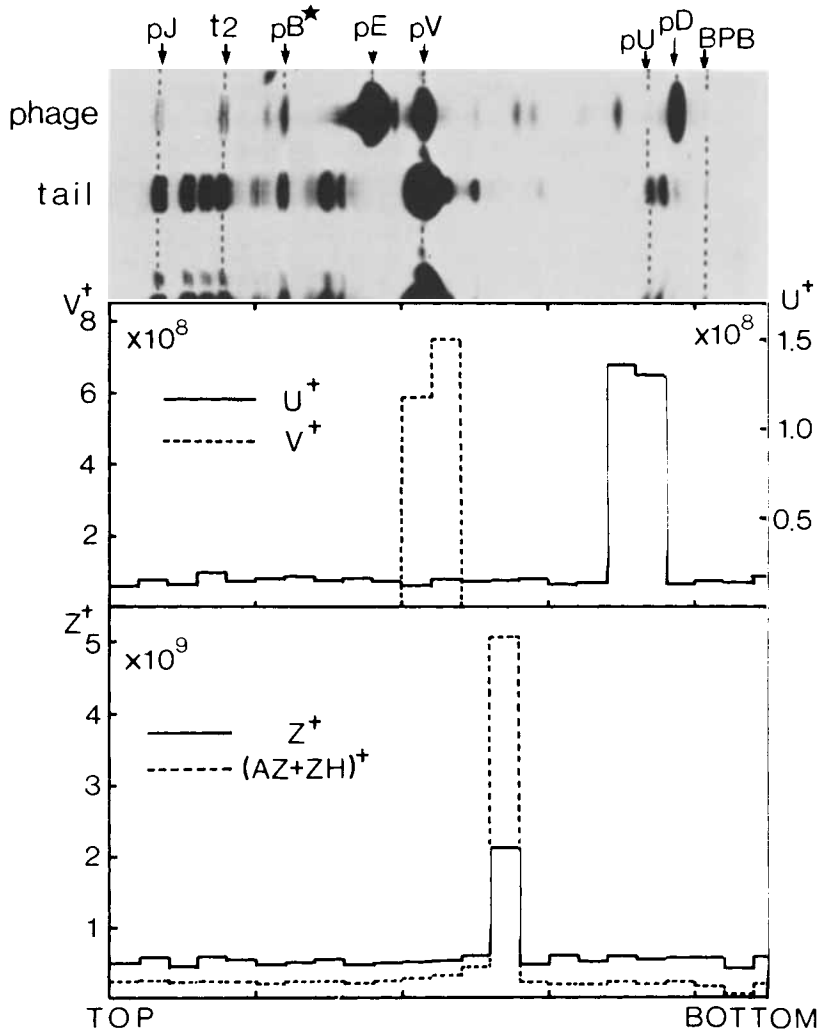


Fig. 7. In vitro complementation activities extracted from SDS gel after electrophoresis of purified tails. The top part of the figure shows the part of the gel stained with Coomassie Blue. The other part of the gel was cut off before staining and tested for in vitro complementation activities according to Materials and Methods section. The results of in vitro complementation are shown in the middle and the bottom part of the figure. The abscissas of the three parts of the figure are coincident and reflect the original position in the gel.

the protein carrying Z^+ activity is almost the same as that of a candidate of pZ detected by gel electrophoresis based on the polar effect of mutations in gene F on the expression of gene Z (R. Hendrix, personal communication).

DISCUSSION

The experimental results concerning pU can be summarized as follows:

1) Polytails are formed in U^- lysates (8, 10).

2) pU molecules are present in the cell in a larger number than pV molecules, but only a very small amount if at all of pU is incorporated into phage particles or tails (4). We found that pU is present both in phage particles and in tails.

3) Both pU and pV act during the same step of the morphogenetic pathway of the λ tail. Probably pU is necessary throughout the assembly of pV.

4) Free polytails in U^- lysates are activated by pU, bind a head and form long-tailed phage in vitro.

5) Giant polytubes are found in $U^- \cdot H^-$ lysates. It seems that pU prevents the aberrant assembly of pV into the giant polytubes and keeps pV soluble and ready to interact with the initiator when the formation of the initiator is blocked.

6) pV isolated from dissociated purified tails has V^+ activity but no U^+ activity. This pV also requires pU for in vitro complementation.

From these results we can deduce the following properties of pU.

a) pU is not an enzyme which modifies pV covalently. If pU were such an enzyme, pV obtained from tails would not require pU for in vitro complementation.

b) Judging from results 2 and 4 above, pU probably remains at the top of the normal tail and prevents further attachment of pV; and this remaining pU plays a role in the attachment of the tail to the head.

c) The simplest explanation for the "solubilization" of pV by pU would be a complex formation between pU and pV. This would also explain why a large intracellular concentration of pU molecules is observed.

These arguments limit the number of possible models for the mechanism of the action of pU. One of the possible models is as follows:

The 25S precursor consists of the tail fiber, the basal part and a hypothetic fibrous material (probably a protein) which determines the length of the tail. One end of the fibrous material attaches to the top of the basal part and the other end has a site which interacts with pU. During tail formation pV forms a complex with pU which prevents the abortive assembly of pV into giant polytubes even if unassembled pV accumulates in the cell. This (pV + pU) complex assembles on the basal part around the fibrous material. As soon as the complex attaches to the basal part, pU is released from the complex and only pV remains; another (pV + pU) complex attaches to this pV at the next step. Thus the elongation of the tubular part of the tail proceeds complex by complex, releasing pU each time, until the tubular part has reached the end of the fibrous material. Now the last (pV + pU) complex attached to the top of the growing tail interacts with the end of the fibrous material. When this interaction occurs, pU stops being released from the tail and

remains on top of it. This pU prevents further attachment of a complex to the tail and plays a role in the attachment of a head. pV alone can also assemble starting from a 25S initiator, but in the absence of pU the elongation of the tubular part of the tail continues until all pV in the cell has assembled on the 25S precursors used for initiation. We think that polytails are formed in U^- lysates in this way.

The hypothetic fibrous material is dispensable if pV itself can measure the length of the tail as postulated in the "sophisticated cummulated strain model" by Kellenberger (15). In this case the model would be as follows: cummulated strain induces a conformation change of pV molecules in the tail when pV polymerizes up to the tail's correct length and this conformation change makes pU unable to detach from the tail.

The essential points common to both models are as follows:

1) pU is a termination factor for the assembly of pV as well as a solubilization factor for pV.

2) Assembly proceeds by a (pV + pU) complex; pU is detached after each addition of the complex to the growing tail.

3) At the termination of assembly, the last pU is no more detached and remains on the top of the tail.

The models mentioned above have to be tested experimentally by demonstrating the existence of a (pV + pU) complex and/or of the hypothetical fibrous material.

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