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Biochemistry

Enzymatic breakage of the cohesive end site of phage λ DNA: Terminase (*ter*) reaction

(λ assembly/*cos* cutting/*A* gene product/spermidine/ATP)

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ABSTRACT **An *in vitro* system is described for measuring the endonucleolytic conversion of the phage λ cohesive end sites in concatemeric DNA to the cohesive chromosomal ends of the mature molecule. This enzymic process, known as the *ter* reaction, is catalyzed by purified λ *A* gene protein. The reaction is markedly stimulated by ATP, Mg^{2+}, spermidine, and one or more uncharacterized factors present in extracts of uninfected *Escherichia coli* cells. *In vitro*, the *ter* reaction proceeds in the absence of proheads under conditions that are similar to those previously found necessary for the formation of a DNA·*A* gene protein intermediate for the initiation of packaging.**

The topological forms through which the chromosome of coliphage λ alternates during its life cycle are understood in considerable detail. Mature λ DNA, as extracted from the phage, is a linear duplex of some 47,000 uniquely ordered base pairs (1). At each end of the molecule, the 5′-terminated chains protrude as single strands of 12 nucleotides (2, 3). The left protrusion is complementary to the right one, so that the ends of the chromosome can anneal, converting the linear molecule to a ring form (4). Indeed, *in vivo*, when λ DNA is injected into a sensitive bacterium, circularization follows rapidly (5, 6) and the two terminal phosphodiester bond interruptions are closed by ligase action (7). The site of this terminal joint, now an uninterrupted duplex, is called *cos*, for cohesive-end site (8).

In the lytic mode of infection, λ DNA circles replicate, first by giving rise to daughter duplex circles (9). Later during infection, the rolling-circle mode of replication takes over (10), and concatemers of λ chromosomes are generated (11–15). These concatemers are the substrates from which monomeric chromosomes are cut and packaged into preformed proheads (8, 16, 17). The cutting event is known as the *ter* (for terminase) reaction (18). In this reaction, the single-stranded ends of the λ chromosome are regenerated by the introduction of two specific single-strand breaks, staggered twelve nucleotides apart, on opposite strands of the *cos* site.

The *ter* reaction is intimately coupled to DNA packaging into proheads. When mutations in λ genes *E*, *B*, *C*, or *Nu3* or the host gene *groE* are present and competent proheads are therefore not made, *cos* site cleavage is not observed *in vivo* (19, 20, 14). Mutations in three other λ genes—*Nu1*, *A*, and *FI*—similarly lead to an accumulation of uncut concatemers, even though competent proheads are present (13, 21, 22). These latter three functions, therefore, are believed to participate in the interaction of DNA and proheads, initiating or promoting the packaging reaction, or catalyzing cleavage at the *cos* sites.

Wang and Kaiser (23) showed that *cos* site cleavage could by catalyzed *in vitro* by crude extracts of λ A^+, but not by λ A^--infected cells, implicating the *A* product (pA) as an essential phage-specific component of the *ter* system. Furthermore, this reaction could take place in the absence of proheads, showing that cutting and packaging could be uncoupled in the cell-free system. Other biochemical studies on λ head assembly *in vitro* (17, 24, 25) have suggested the following sequence of events. Concatemers of λ DNA and pA interact under appropriate conditions to form a DNA·pA complex. To this complex, a source of proheads can be added, with the resulting formation of a DNA·pA·prohead packaging intermediate. Upon addition of pD, pW, and pFII, DNA-filled heads are completed and stabilized and tails can join to give λ plaque-forming units (PFU).

The ability to conduct the overall packaging reaction in stages (21, 25) and to add each of the known morphogenetic gene products for head assembly in sequence has now allowed us to demonstrate that mature chromosomal ends appear upon exposure of DNA concatemers to highly purified pA, even before proheads are added. Furthermore, the number of ends measured after reaction with pA is as great as that measured at the end of the overall assembly reaction when completed phage are present.

MATERIALS AND METHODS

Unless indicated otherwise, the materials and procedures used here have been described before (21, 25, 26). This includes the bacterial strains used, media and buffers, the preparation of mature and immature carbon-14-labeled λ DNA; and the preparation of pA, proheads, and crude extracts for executing the cell-free λ assembly reaction in stages.* Agarose (type II: medium EED) for electrophoresis of DNA was from Sigma. *Eco*RI restriction endonuclease (1.5×10^5 units per ml) was from Miles Laboratories and was used without dilution, as outlined below. Adenylyl methylenediphosphonate analogues of ATP were purchased from Miles Biochemicals or HPLC Biochemicals.

Assays for *Cos* Site Cleavage. *Cos* site cleavage was catalyzed under conditions used previously to form a complex between pA and λ DNA concatemers for initiation of packaging (25). Five to ten microliters of ^{14}C-labeled λ DNA concatemers (0.5–1.5 A_{260} units/ml, $4–8 \times 10^6$ cpm/ml) was added to 40 μl of buffer A. This was followed by the addition of 4 μl of a solution containing 6 mM Tris·HCl (pH 7.4), 15 mM ATP, 18 mM $MgCl_2$, 60 mM spermidine·HCl, and 30 mM 2-mercaptoethanol. Reaction was then initiated by the addition of 20 μl of a mixture of a sonicated extract of uninfected or λ-infected *Escherichia coli* cells (final protein concentration, 20–40

Abbreviations: pA, pD, the protein products of gene *A*, gene *D*, etc.; PFU, plaque-forming units.

* The immature λ DNA was isolated as randomly broken molecules, of average length 2 to 3 chromosomal units, from cells infected with maturation-defective (A^-) phage (25). The pA preparations used were approximately 20% pure as judged by electrophoresis in sodium dodecyl sulfate/polyacrylamide gels.

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mg/ml) and purified pA. Usually 5 parts by volume of the sonicate were mixed with 1 part of pA so that the input of pA was 10 units per reaction tube. Reaction was for 15 min at 20°. Ten microliters of 0.5 M EDTA was then added to stop the reaction, and this was followed by the addition of 2.0 ml of a solution containing 10 mM Tris·HCl (pH 8.6), 100 mM NaCl, and 2 mM EDTA. Two milliliters of freshly distilled phenol saturated with 10 mM Tris·HCl (pH 8.6) was then added, and the DNA was extracted by gentle rolling of the mixture for 30 min. The aqueous, DNA-containing phase (≈2 ml) was removed, and the nucleic acids were precipitated overnight at −20° after the addition of 4 ml of 95% ethanol; this was followed by centrifugation for 30 min at 27,000 × *g*, 5°. The supernatant was discarded and the centrifuge tubes were drained carefully. The precipitate was washed with 70% ethanol at 5° and recentrifuged. The supernatant was discarded, the tubes were drained, and the small amount of precipitate was dissolved in 100 μl of a solution containing 15 mM NaCl, 10 mM $MgCl_2$, and 50 mM Tris·HCl (pH 7.4). Dissolution was aided by agitating the tubes in a 50° water bath. A 5-μl sample was removed for counting in a liquid scintillation spectrometer to assess the recovery of the radiolabeled DNA substrate (usually 60–80%). Samples of the preparation were then processed in the following two ways to assay for cohesive ends.

(*i*) One method of assay was biological, using the helper-dependent transfection system of Kaiser and Hogness (27). In this assay, uptake of λ DNA by helper phage-infected cells depends specifically upon the presence of cohesive ends in the transforming DNA that are homologous to those of the helper. Here, the helper-infected cell complexes were QD50003(λi^{21}) bacteria superinfected with $\lambda i^{21} Sam_7$ phage. A 10-μl sample of the concatemeric imm^{λ} DNA treated as above was diluted serially into TCM buffer (27) prior to its addition to the competent cells.

The transfection assay is, therefore, a marker-rescue test involving the imm^{λ} marker on the DNA substrate and the imm^{21} genotype of the helper. Fragments of λ DNA less than one chromosome long, but containing one cohesive end in continuity with the immunity region, would be expected to register as plaques in the system (28). In a related context, however, the efficiency of plating of longer than unit molecules—for example, of a concatemer containing three or four *cos* sites, one of which has been broken *in vitro*—is not known. A reduction in the sensitivity of this assay as a measure of *cos* sites broken may be expected from the exposure of the DNA to the crude components present in the cell-free *ter* reaction. It is known that about four single-strand breaks per mature λ DNA molecule constitute an inactivating event in this transfection assay (29). In our hands, purified mature λ DNA gives 10^7–10^8 plaques per μg. If this DNA is exposed to the conditions of the *ter* reaction (with or without added pA), followed by phenol extraction, precipitation, and dissolution, the specific infectivity is reduced to 10^5–10^6 plaques per μg of DNA recovered. The specific infectivity of the immature concatemers successfully reacted in the cell-free *ter* system often approaches this value. Concatemers not treated with pA give 10^2 or fewer plaques per μg, which constitutes the background of the assay.

(*ii*) *Ter* break production was also measured by a physical assay involving *Eco*RI restriction analysis according to the following plan. *Eco*RI nuclease makes five cuts in a mature λ DNA to give six fragments—A to F, named in their order along the λ map from left to right. Thus, the terminal fragments are called A and F and correspond, respectively, to the left and right ends of the molecule (30). It may be expected that the restriction pattern of randomly broken concatemers of λ DNA (13) would not show the terminal A and F pieces due to their covalent connection at *cos*; rather, a larger F–A fusion fragment might be observed. Following *cos* site cleavage on both strands of the DNA, the F and A pieces should reappear. The degree of *cos* site cleavage can be estimated from a single electropherogram by calculating the ratio of the intensity of the F (or A) fragment that appears to the intensity of a fragment from the interior of the molecule—the B fragment, for example. This F/B value can, in turn, be compared to the fixed F/B value for mature λ DNA. In mature λ DNA the expected F/B ratio is 0.7, based on mass (30). For the mature λ DNA preparations labeled *in vivo* with [^{14}C]thymine that were used in these studies, F/B was 0.68 ± .02, as estimated by radiointensity. A similar assay for *cos* site cleavage *in vivo* has been used by Reuben and Skalka (31).

Accordingly, *Eco*RI (1 μl) was added to 50 μl of the DNA extracted as above following the *ter* reaction. Incubation was for 30 min at 37°. The reaction was stopped by adding 10 μl of a solution containing 100 mM EDTA, 5% sodium dodecyl sarcosinate, 0.02% bromphenol blue, and 50% (vol/vol) glycerol. The mixture was heated for 5 min at 70° and cooled rapidly on ice. Electrophoresis of the DNA in agarose was performed in a horizontal slab apparatus (Aquebogue Machine and Repair Shop) described by McDonell *et al.* (32). The borate-containing buffer system of Mizuuchi and Nash (33) was used. Agarose concentration was usually 0.8%, but 0.5% gels were also used to show the difference between the A and the fused FA fragments more clearly. Electrophoresis was for 16–18 hr at 25 V. Following electrophoresis, the gel was dried on a sheet of filter paper and exposed to Kodak SB-5 x-ray film for autoradiography. Exposure was for 5–10 days prior to development. X-ray films exposed within the gamma range of their response could be scanned densitometrically using a linear transport device coupled to a Gilford spectrophotometer. [Staining of wet agarose gels with ethidium bromide as an alternative to autoradiography is possible after removal of nucleic acids from the sonicated extracts that are included to provide host factors in the *ter* reaction (unpublished observations).]

Fig. 1 illustrates how the biological and physical assays are used to measure *cos* site cleavage. The data show a comparison of the infectivities and restriction patterns of (a) mature λ DNA; (b) immature λ DNA concatemers, and (c) concatemers cleaved to a moderate degree in a pA-dependent, cell-free *ter* reaction.

RESULTS

Cos Site Cleavage in Relation to Packaging _In Vitro_. In order to determine the point in the *in vitro* packaging reaction at which the *ter* breaks are made, assembly was staged as follows (25). (*i*) ^{14}C-labeled concatemers of λ DNA, spermidine, Mg^{2+}, and ATP were mixed. (*ii*) To this mixture was added pA together with a sonicate of uninfected *E. coli* cells; incubation followed to allow the formation of a DNA·pA complex. (*iii*) Proheads were then added and the mixture was incubated further for the purpose of forming a DNA·pA·prohead packaging intermediate. (*iv*) Finally, a pA-negative, prohead-negative extract of λ*AamBam*-infected cells was added to provide pD, pW, pFII, and tails, and the mixture was further incubated. At the end of each step, a sample of the reaction mixture was removed and assayed for breakage at *cos* sites in the exogenous radiolabelled DNA substrate both by the helper-dependent transfection method and by *Eco*RI restriction analysis. The number of assembled PFU was also measured after steps *iii* and *iv*. The results of this experiment are given in Fig. 2. The data show that a maximal level of *cos* site cleavage

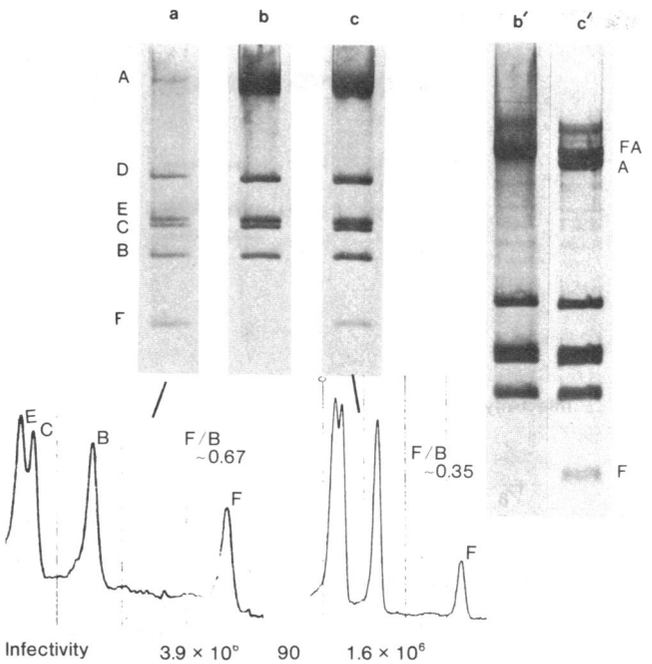

FIG. 1. Physical and biological assays for *cos* site cleavage. Autoradiograms of *Eco*RI restriction patterns are given for (a) mature λ DNA; (b) immature λ DNA concatemers; (c) concatemers cleaved in a *ter* reaction in which purified pA was supported by a sonicate of induced 594(λ*Aam*$_{a19}$ *Dam*$_{15}$*cI*$_{857}$*Sam*$_7$) cells. Electrophoresis was in 0.8% agarose for a, b, and c; and again in 0.5% agarose, shown for the b and c samples (b′ and c′, respectively). Migration was from top (cathode) to bottom (anode). Densitometric scans showing the bottom four bands in a and c are shown below. F/B is the ratio of the areas of the F and B bands, respectively, as determined from the scans. Infectivities given correspond to the samples analyzed in a, b, and c, and are expressed in PFU per μg of DNA recovered after the *ter* reaction, phenol extraction, and reprecipitation.

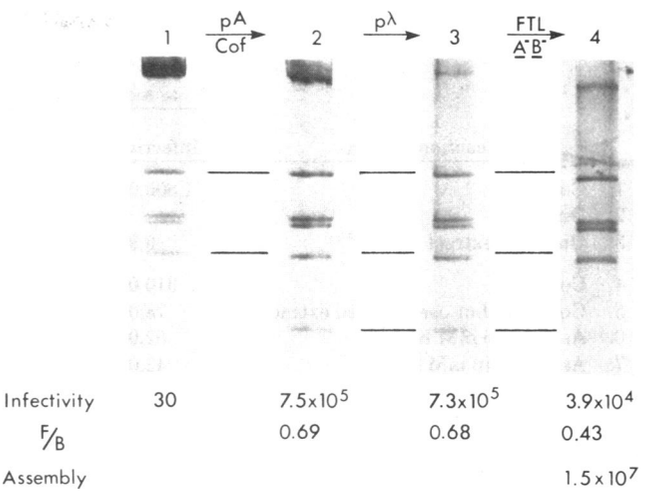

FIG. 2. Sequential analysis of the concatemer DNA substrate during the staged packaging reaction. The analyses shown are as for Fig. 1 with electrophoresis in a 0.8% agarose gel. ^{14}C-Labeled concatemeric DNA (25 μl) was mixed with 150 μl of buffer A and 20 μl of the ATP, Mg^{2+}-, spermidine-containing mixture. A 40-μl sample of this mixture was removed for the restriction and infectivity analyses (channel 1). To the remainder, 80 μl of a mixture of pA and a sonicated extract of uninfected 594 cells (Cof, for cofactors) was added. After incubation for 10 min at 20°, a 60-μl sample was removed for analysis (channel 2). Fifteen microliters of purified proheads (pλ, for petite λ) (21) was then added and incubation was continued (10 min, 20°), after which time a 65-μl sample was removed for analyses (channel 3). Assembled PFU were not detectable at this point. Finally, 400 μl of an FTL extract (21) of *AamBam*-infected cells was added to catalyze completion of phage assembly; incubation was for 45 min at 20°. A 260-μl sample was removed for analyses, including plating for assembled phage (channel 4). Assembly is expressed as PFU per μg of DNA added.

is achieved at the outset of the assembly pathway, upon interaction of DNA with pA, even in the absence of proheads. Upon reaction with pA and then with proheads (steps *ii* and *iii*), the infectivity of the DNA remains maximal and the *Eco*RI restriction pattern appears unperturbed. These results, therefore, establish that *cos* site cleavage can proceed at high efficiency in the absence of proheads under conditions that are optimal for subsequent packaging into proheads (25). The data also suggest a possible reason why such a small fraction of the input chromosomes are converted to PFU. Upon exposure to the FTL extract (step *iv*), during which time phage formation is completed, there is a 20-fold loss of infectivity. This loss of infectivity is accompanied by a decrease in the molecular weight of the terminal F fragment, together with a dispersion in its size. Thus, it appears that a large fraction of the DNA made mature at the outset suffers nucleolytic attack when exposed to extracts added to catalyze the final steps of viral assembly.

Requirements. Having shown that the *ter* reaction can proceed in the absence of proheads, we set out to examine its requirements. As indicated above, the reaction proceeds under conditions in which a pA·DNA packaging intermediate can be formed (25). These include pA, an extract of *E. coli* cells, spermidine, Mg^{2+}, and ATP. In Table 1, lines 1 and 4, we show again that a high level of *cos* site cleavage is observed when all of these reagents are present. When pA is omitted from the reaction, there is no evidence of *cos* site cleavage either in the *Eco*RI restriction pattern or in the infectivity assay (Table 1, line 2). When the extract of *E. coli* cells is omitted, the physical assay fails to register evidence of *cos* site cleavage, but the more sensitive biological assay shows that *ter* breaks are made in a yield that is reduced to 1/250th compared to that attained in the complete system (line 3). We interpret these results to mean that pA is an absolute requirement for *cos* site cleavage, but that one or more factors present in uninfected *E. coli* cells greatly stimulate the reaction. The residual *ter* activity observed with purified pA in the absence of the host factors remains constant through the final steps of purification of this protein, which include sequential chromatography on DEAE-Sephadex, Bio-Rex-70, and Sepharose. Although a persistent contamination of pA by the host-derived cofactor(s) cannot be excluded, the low level of *ter* activity may be an intrinsic property of the *A* protein. Such an interpretation implicates pA itself as the *ter* nuclease.

Next, we asked whether the contribution of the cell extract was in the form of a small molecule or a macromolecule. Accordingly, a sonicate of uninfected cells was dialyzed extensively and tested for its ability to stimulate pA in the *ter* reaction. The activities of the undialyzed and dialyzed extracts are also included in Table I (lines 4 and 5) for comparison. Dialysis of the extract results in a reduction to 1/4th in the yield of *ter* breaks produced. In other experiments, the reduction observed was to as low as 1/20th. Although we have not isolated and identified the factor that is lost upon dialysis, we find that it can be replaced by monovalent cations. Table 1 gives the results obtained when the dialyzed extract is supplemented with various concentrations of KCl (lines 6–10). At about 50 mM KCl, final concentration in the reaction, the system is fully reconstituted. Na^+ and NH_4^+ salts also restore the activity (data not shown). Salt, however, is not the only factor involved in the cell-extract effect. When KCl at the optimal concentration is

Table 1. Requirements in production of ends

	Reaction system	Yield, as measured by Infectivity	*Cos* sites broken
1.	Complete	1,800,000	0.54
2.	Omit pA	90	NM
3.	Omit cell extract	6,900	NM
4.	Complete	310,000	0.37
5.	Complete but use dialyzed extract	78,000	0.12
6.	As in 5, +5 mM KCl	52,000	0.13
7.	As in 5, +10 mM KCl	42,000	0.05
8.	As in 5, +20 mM KCl	110,000	0.16
9.	As in 5, +50 mM KCl	480,000	0.36
10.	As in 5, +100 mM KCl	100,000	0.17
11.	Omit dialyzed extract + 50 mM KCl	8,100	NM
12.	Complete but use dialyzed extract + 50 mM KCl	1,800,000	0.57
13.	As in 12, but omit spermidine	450	NM
14.	As in 12, but omit ATP	270	NM
15.	As in 12, but omit Mg^{2+}	290,000	0.08
16.	As in 12, but add EDTA to 20 mM	350	NM

Where indicated, pA-dependent *ter* break production was supported by a sonicated extract of uninfected 594 cells, or by the same extract dialyzed for 18 hr against 10^3 vol of buffer A. For line 15, the extract was dialyzed against buffer A lacking Mg^{2+}; such extracts are fully active if Mg^{2+} is included again in the reaction. Infectivities given are expressed as PFU per μg of DNA recovered after reaction, phenol extraction, and reprecipitation. The fraction of *cos* sites broken was estimated by restriction analysis from the ratio of F/B for the reacted concatemeric substrate to F/B for the mature λ DNA reference taken as 0.7. NM, not measureable. Lines 1–3, 4–11, and 12–16, represent three different experiments.

included with pA in the reaction, but the dialyzed extract is omitted, there is a reduction to 1/500th in the extent of *ter* activity (Table 1, line 11). Together, these results indicate that monovalent cations support pA-dependent *cos* site cleavage but that one or more other undialyzable reactants present in *E. coli* extracts greatly stimulate the system.

Other requirements were determined by using a dialyzed extract of uninfected cells supplemented with KCl to support the pA-dependent reaction. Various compounds were omitted, one at a time, from the mixture (Table 1). Omission of spermidine (line 13) or ATP (line 14) reduced reaction to background levels. Similarly, addition of excess EDTA to the complete system virtually eliminated *cos* site cleavage (line 16). Simple omission of $MgCl_2$ reduced the yield of breaks by almost one order of magnitude (line 15).

As a first step in clarifying the role of ATP, we asked if either of the two ATP analogues 5′-adenylyl-α,β-methylenediphosphonate (Ado*P*[CH_2]*PP*) and 5′-adenylyl-β,γ-methylenediphosphonate (Ado*PP*[CH_2]*P*) can substitute for ATP in the *ter* reaction. As shown in Fig. 3, Ado*P*[CH_2]*PP*, but not Ado*PP*[CH_2]*P*, is effective as a cofactor or substrate. These results imply that the recognition or cleavage of the β–γ pyrophosphate bond is an important feature of the *ter* endonuclease system.

Note on DNA Substrate Specificity. Prohead-independent *cos* site cleavage, as assayed in these studies, shows little specificity in respect to the long-range topology of λ DNA. For example, the *cos* sites present on covalently closed circles of λ DNA are readily broken. Furthermore, concatemers of λ DNA can first be fragmented by *Eco*RI action; the *cos* sites present on the FA segments can then be broken by exposure to pA under standard conditions (data not shown).

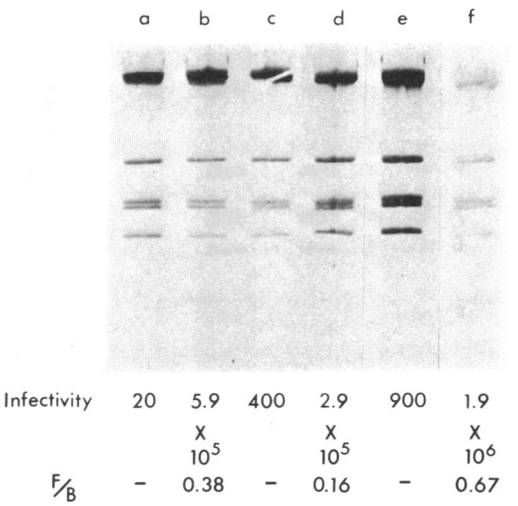

FIG. 3. Effect of ATP and its methylene analogues on *cos* site cleavage. The pA-dependent *ter* reaction was supported by an extract of strain 594 cells that had been dialyzed for 18 hr against 10^3 vol of buffer A. Reaction was in the presence of 50 mM KCl. ATP, 5′-adenylyl-α,β-methylenediphosphonate, and the β,γ-methylene counterpart, where present, were at 1 mM. (a) Immature λ DNA substrate pattern; (b) complete system; (c) complete system minus ATP; (d) complete system minus ATP plus the α,β-methylene analogue; (e) complete system minus ATP plus the β,γ-methylene analogue; (f) mature λ DNA pattern. Electrophoresis was in a 0.8% agarose gel.

DISCUSSION

The biochemical results presented indicate that the following reaction has been studied:

$$\lambda\ cos\ \text{DNA} + \text{pA} \xrightarrow[\text{spermidine, factors}]{\text{ATP},Mg^{2+},M^{+},} \lambda\ \text{cohesive ends·pA}.$$

The scheme postulates that after catalyzing *cos* site cleavage the *A* product is retained at the site of breakage to serve the further functions of prohead binding and the nucleation of DNA packaging (25).

Although prohead-independent *cos* site cleavage may allow the study of an important mechanism in isolation, why the *ter* reaction should proceed *in vitro* in the absence of packaging will have to be explained. Because *in vivo* no maturation takes place without proheads, the paradox may invite a more intensive search for a coupling element that, so far, has not been revealed in simple mixing experiments involving pA and various cell extracts. Alternatively, it is possible that *cos* site cleavage by pA can proceed in the absence of proheads *in vivo*, but that the product of this reaction is unstable and a reversing process that leads to intact *cos* sites dominates. Packaging into proheads may drive the *ter* reaction irreversibly toward the cut state of *cos*.

The question then arises whether all or some of the *cos* sites that are cleaved *in vitro* in the pA-dependent reaction in the absence of proheads are representative of the DNA·pA complexes that will initiate packaging when proheads are added, ultimately giving rise to PFU. Although the conversion of *cos* sites to ends as measured by the restriction assay can sometimes be virtually quantitative (see Fig. 2), the question cannot be answered easily when the subsequent assembly reaction registers only about 10^{-3} of the chromosomal input as PFU. Thus, it remains possible that the DNA molecules that enter PFU are, in fact, ones to which pA has bound but in which no *ter* cuts have been made. We would argue, however, from circumstantial evidence that prohead-independent *cos* site cleavage and DNA·pA complex formation likely are aspects of one and

the same reaction. Because mature λ DNA is packaged *in vitro* in a pA-dependent reaction (24, 25) there is little reason to believe that λ DNA concatemers made mature by prior pA action are not the ones that proceed to PFU when the other morphogenetic reactants are provided. This is not to deny that productive DNA·pA complexes, in which the *cos* sites are not immediately cut, may also be formed in this system.

In a formal sense, the designation of pA as the *ter* endonuclease might still be regarded with some caution in view of recent studies on the role of the λ *Nu1* gene (21; R. Weisberg and N. Sternberg, personal communication). The *Nu1* gene, as defined by a defective missense mutation (*t16*) in λ and by a corresponding amber mutation in phage φ80 (34), maps between the left chromosomal end and gene *A*. The *Nu1* mutant of λ is phenotypically A^- *in vivo*, as well as by *in vitro* tests for DNA packaging. Furthermore, *ter* activity cannot be detected in, or isolated from extracts of λ $Nu1^-$-infected cells (unpublished observations). Accordingly, one plausible hypothesis for pNu1 action is that it modifies and activates the *A*-specific polypeptide both for its packaging and *ter* functions. One simple model for such an activation, in turn, might feature the *Nu1*- and *A*-specific polypeptides associating to form the native protein that we purify and designate as pA. If such a situation in fact exists, then the endonucleolytic active center of the complex could reside in either pNul or pA, or encompass both polypeptide chains.

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