Linearization of baculovirus DNA enhances the recovery of recombinant virus expression vectors

Paul A.Kitts*, Martin D.Ayres and Robert D.Possee
NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR, UK

Received July 26, 1990; Revised and Accepted September 3, 1990

ABSTRACT

Engineered derivatives of *Autographa californica* multiple nucleocapsid nuclear polyhedrosis virus (AcMNPV) possessing a unique restriction site provide a source of viral DNA that can be linearized by digestion with a specific endonuclease. Circular or linearized DNA from two such viruses were compared in terms of their infectivity and recombinogenic activities. The linear forms were 15- to 150-fold less infectious than the corresponding circular forms, when transfected into *Spodoptera frugiperda* cells using the calcium phosphate method. Linear viral DNA was, however, proficient at recombination on co-transfection with an appropriate transfer vector. Up to 30% of the progeny viruses were recombinant, a 10-fold higher fraction of recombinants than was obtained from co-transfections with circular AcMNPV DNA. The isolation of a recombinant baculovirus expression vector from any of the AcMNPV transfer vectors currently in use can thus be facilitated by linearization of the viral DNA at the appropriate location.

INTRODUCTION

Baculovirus expression vectors derived from *Autographa californica* multiple nucleocapsid nuclear polyhedrosis virus (AcMNPV) and *Bombyx mori* MNPV have been widely employed to produce high levels of accurately processed, and biologically active proteins (1–5). Due to the large size of the baculovirus genomes (about 130 kbp) (1, 3) and the frequency of restriction endonuclease sensitive sites, it is difficult to insert foreign genes into the viral DNA directly. The preferred route to obtain a recombinant virus is to clone an appropriate segment of the viral DNA into a bacterial plasmid to create a transfer vector (6). The sequences in such transfer vectors are then manipulated to introduce a foreign gene under the control of a proficient viral promoter and produce a recombinant transfer vector. In this vector, the foreign DNA is flanked by baculovirus DNA sequences. DNA from the modified transfer vector is then introduced into insect cells together with infectious viral DNA. Inside the cells, homologous recombination occurs whereby a portion of the wild-type viral DNA is exchanged for the corresponding sequences in the transfer vector, generating a recombinant virus. Since the frequency of such recombination events is typically 0.1 to 1% (4, 7), the identification of a recombinant virus against a background of parental viruses is often a very tedious step. In view of the increasing interest in the use of baculovirus expression vectors, we have sought ways to increase the percentage of recombinants among the progeny viruses.

In yeast and mammalian cells, DNA molecules containing double-strand breaks are highly recombinogenic (8, 9) and in these systems linearization of the incoming DNA is routinely used to promote recombination with homologous sequences in the chromosome (9, 10). To our knowledge, however, the relative recombinogenicity of circular and linear forms of DNA in insect cells had not been studied. It is known that linear fragments of AcMNPV DNA can recombine with intact viral DNA when co-transfected into either *Spodoptera frugiperda* cells, as used to map mutations in AcMNPV by marker rescue (11, 12), or *Galleria mellonella* larvae (13). It has also been reported that the linear form of baculovirus DNA is not infectious (14). Based on these reports, it seemed plausible that linearized baculovirus DNA might be recombinogenic but not infectious.

For these reasons, we reexamined the infectivity of linearized viral DNA and investigated the ability of the linear DNA to recombine with transfer vectors. In comparison to circular viral DNA, the linear form has a greatly reduced infectivity but yields a higher proportion of recombinant viruses from co-transfections with the appropriate transfer vectors. This finding can be exploited to facilitate the construction of baculovirus expression vectors.

MATERIALS AND METHODS

Viruses and cells

AcMNPV C6 (15) and its derivatives AcUW1-*lacZ* (16) and AcRP6-SC (this work) were propagated in *Spodoptera frugiperda* cells (IPLB-SF-21) (17) at 28°C using TC100 medium (Gibco) supplemented with 5% or 10% fetal calf serum (FCS). AcMNPV viral DNA was isolated as described (15).

Plasmids

pAcRP6 (18) and pAcRP23-*lacZ* (19) have been previously described. pAcUW1-NA has the N2 neuraminidase gene from

* To whom correspondence should be addressed

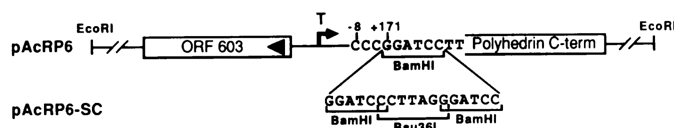

Figure 1. Diagram of the AcMNPV DNA in the transfer vectors pAcRP6 and pAcRP6-SC. pAcRP6 is a transfer vector based on a derivative of the AcMNPV EcoRI-I fragment (15) in which polyhedrin sequences from −7 to +170 have been replaced by sequences from a BamHI linker (18). (The numbering system is based on the sequence of the wild-type polyhedrin gene (36, 37) with the initiation codon representing nucleotides +1, +2, +3 and upstream nucleotides having negative number assignments). The locations of the polyhedrin transcription start site (T), the residual carboxy-terminus of polyhedrin and the upstream open reading frame (ORF 603; 38) are shown. pAcRP6-SC was derived from pAcRP6 by insertion of a BamHI-Bsu36I adaptor oligonucleotide into the BamHI site creating the sequence shown.

influenza A/NT/60/68 (20) inserted into the BglII site of pAcUW1 (16) such that neuraminidase is expressed from the p10 promoter (A. Cartwright, personal communication).

Construction of AcRP6-SC

A synthetic BamHI-Bsu36I adaptor oligonucleotide (5′pGATCCCCT[A,T]AGG3′) was inserted into the unique BamHI site of pAcRP6 (18; Figure 1). The sequence of the insert in one of the resulting plasmids, pAcRP6-SC, was determined by the Maxam and Gilbert method (21) and is shown in Figure 1. pAcRP6-SC plasmid DNA and infectious AcMNPV C6 DNA were co-transfected into *S. frugiperda* cells and a polyhedrin-negative recombinant virus was isolated and plaque purified by standard procedures (22). The resulting virus, AcRP6-SC (Single Cut), has the BamHI-Bsu36I-BamHI linker in place of part of the polyhedrin gene (Figure 1).

Linearization of AcMNPV DNA

To prepare linear AcRP6-SC or AcUW1-*lac*Z DNA, 1 μg of viral DNA was digested with 5 units of Bsu36I endonuclease (New England Biolabs) for 2 hr at 37°C in 50 μl of the recommended buffer (10 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol and 100 μg/ml bovine serum albumin). The enzyme was inactivated by heating to 70°C for 15 min. and the digest stored at 4°C. Mock digested circular viral DNA was prepared by the same procedure except that Bsu36I endonuclease was omitted. Conversion of the DNA from the circular form to the linear form was monitored by electrophoresis of 20 ng of the digested DNAs on 0.3% agarose minigels (7.5 cm) in 1xTBE (89 mM Tris, 89 mM borate, 2 mM Na_2EDTA, pH 8.3) containing 0.5 μg/ml ethidium bromide at 40 V for 3 hr. The location of Bsu36I cleavage was mapped by adding 0.5 unit of BglII (Boehringer Mannheim) to 200 ng of either Bsu36I digested viral DNA or untreated DNA in 10 μl Bsu36I buffer, incubating at 37°C for 2 hr, and electrophoresing 170 ng of the digestion products on a 0.3% agarose gel at 5 V for 16hr.

For experiments comparing different treatments to the viral DNA, the digestion of AcRP6-SC DNA was scaled up 3.3-fold. After incubation with Bsu36I, two 54 μl aliquots were removed, heated to 70°C for 15 min. and then stored at 4°C. 5′ phosphate groups were removed from the ends of the linearized viral DNA in the remaining aliquot by addition of 1 unit of calf intestinal alkaline phosphatase (CIP; Boehringer Mannheim), incubation at 37°C for 1 hr and 70°C for 15 min before storage at 4°C. A mock digested sample was prepared in parallel using the same protocol except that the Bsu36I and CIP were omitted. A 6-fold over-digested sample was prepared by digesting 1 μg of viral DNA with 15 units of Bsu36I for 2 hr at 37°C, adding another 15 units of enzyme and incubating for a further 2 hr before heat inactivation and storage.

Assay for the infectivity of AcRP6-SC DNA

Mock digested circular AcRP6-SC DNA and viral DNA linearized by digestion with Bsu36I were prepared as described above. 50 μl of the digests, containing 1 μg of viral DNA, were transfected into *S. frugiperda* cells using a modification of the calcium phosphate precipitation method (23) similar to that previously described (18) except that no plasmid DNA was added. After 2 days at 28°C, the cells and medium were harvested, the volume measured and the virus titrated using a standard plaque assay procedure (24). After 4 or 5 days at 28°C, the plaques were stained with 100 μg/ml Neutral Red for 2 hr and counted. The total yield of virus from 1 μg viral DNA was calculated from the virus titre and the volume of medium recovered from each transfection.

Assay for recombination between AcMNPV DNA and transfer vectors

At the polyhedrin locus

Mock digested, or digested, or digested and dephosphorylated, or over-digested AcRP6-SC DNA were prepared as described above. 50 μl of each sample, containing 1 μg of DNA, or 1 μg of untreated viral DNA made up to 50 μl in 10 mM Tris-HCl, pH 8.0, 0.1 mM Na_2EDTA, were mixed with 5 μg of caesium chloride purified pAcRP23-*lac*Z plasmid DNA and used to transfect *S. frugiperda* cells as described above. Immediately before transfection, one aliquot of the digested DNA was heated to 70°C for 15 min. Viruses produced from the transfected cells were titrated as above except that the plaques were stained with 50 μg/ml Neutral Red and 250 μg/ml X-gal (Sigma) at 28°C for 5−10 hr. The liquid overlay was removed and the plates left at ambient temperature overnight before the blue and white plaques were counted. The number of blue plaques was used to derive the titre of recombinant viruses and the number of white plus blue plaques gave the total virus titre. These virus titres were converted to yields per μg of viral DNA after considering the volume of medium recovered from each transfection. The fraction of recombinant viruses was calculated by dividing the yield of blue plaques by the total yield.

At the p10 locus

Using the same procedure, *S. frugiperda* cells were co-transfected with 1 μg of mock digested circular AcUW1-*lac*Z DNA or 1 μg of viral DNA linearized by digestion with Bsu36I and 5 μg of pAcUW1-NA DNA. Plaque assays were performed on the progeny viruses and the total virus titre determined by staining plaques with Neutral Red. The proportion of recombinant viruses in the progeny was determined as follows: the wells of 96-well microtitre plates were seeded with 1×10^4 *S. frugiperda* cells in 0.2 ml TC100 plus 10% FCS and after overnight incubation at 28°C, each well was inoculated with an individual plaque picked at random from the co-transfection plaque assay. 80−100 plaques were picked from each co-transfection. After incubation at 28°C for 5 days, the medium was removed and the cells in each well were washed with 0.2 ml phosphate buffered saline (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH

7.3). Assays for neuraminidase activity were then performed using the method of Barrett and Inglis (25) but scaling down the volumes 10-fold. At the end of the incubation at 100°C, the colour of the test samples was compared to that of negative controls from uninfected wells. Samples that produced a pink colour indicative of neuraminidase activity were scored as derived from plaques containing a recombinant virus.

RESULTS

A derivative of AcMNPV containing a unique site for a restriction endonuclease

In order to study the infectivity and recombination potential of linear baculovirus DNA, we required a convenient source of linear viral DNA. Previous studies had used controlled shearing, partial digestion with S1-nuclease, or heat treatment of polyhedra to generate linear baculovirus DNA (14, 26). We rejected these methods due to the difficulty in limiting the damage to a single double-strand break. Instead, we constructed a derivative of AcMNPV with a unique site for a particular restriction endonuclease. By screening a battery of commercially available endonucleases using DNA isolated from AcMNPV C6, one enzyme, Bsu36I, was found that did not cut the viral DNA (DNA from the L1 isolate of AcMNPV was also not cut by Bsu36I)(data not shown). A synthetic oligonucleotide containing the Bsu36I recognition site was inserted into the BamHI site of the transfer vector pAcRP6 (18) to generate the plasmid pAcRP6-SC (Figure 1). Following recombination between pAcRP6-SC and AcMNPV C6 DNAs *in vivo*, a polyhedrin-negative virus was isolated in which the wild-type polyhedrin sequence was replaced by the modified sequences from pAcRP6-SC. This AcMNPV derivative contains a unique site for Bsu36I and was designated AcRP6-SC, for Single Cut. Incubation of the DNA extracted from AcRP6-SC virus particles with Bsu36I, converted the DNA into a faster migrating species (Figure 2a). Subsequent restriction with BglII revealed that treatment with Bsu36I had converted the 15 kbp BglII C fragment of AcMNPV into two fragments of 9.2 kbp and 5.8 kbp (Figure 2b); the sizes of these fragments are those predicted for cleavage in the Bsu36I adaptor of AcRP6-SC which is located 5.83 kbp from the left hand end of the BglII C fragment (22; S.C.Howard, personal communication). This demonstrated that the novel species observed in Figure 2a was indeed linear viral DNA. The AcMNPV derivative we have constructed, AcRP6-SC, thus provides a source of viral DNA which can be linearized at a unique site simply by incubation with an appropriate restriction endonuclease.

The infectivity of linear AcRP6-SC DNA

Before undertaking a study of recombination, we determined the infectivity of linear viral DNA. Circular viral DNA from AcRP6-SC and viral DNA that had been linearized by restriction with Bsu36I were transfected into *S. frugiperda* cells in parallel. After two days, the number of infectious virus particles produced by the transfected cells was determined. From the results of several experiments, it is apparent that linear DNA had approximately 1/15th of the infectivity of circular DNA (Table 1). The considerable variation between experiments was probably due to the difficulty in producing consistent calcium phosphate-DNA co-precipitates and to the variable shear damage suffered by the viral DNA during preparation of the precipitate. The relative infectivity of linearized DNA was even lower if the viral DNA was co-transfected with a five-fold excess of plasmid DNA;

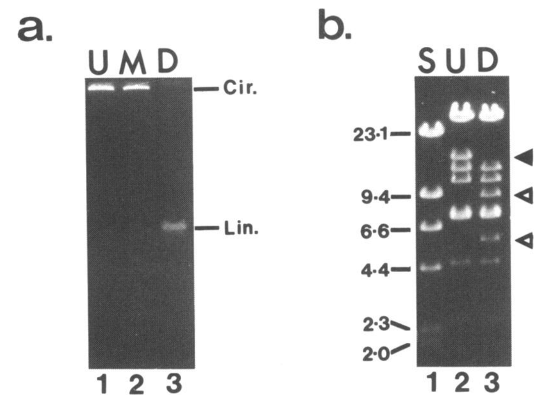

Figure 2. Linearization of viral DNA by digestion with a restriction endonuclease. (a) Agarose gel showing linearization of AcRP6-SC DNA by Bsu36I endonuclease. Lane 1, untreated viral DNA (U); lane 2, viral DNA incubated in the absence of restriction enzyme (mock digested, M); lane 3, viral DNA digested with Bsu36I (D). Under these conditions, circular viral DNA (Cir.) does not enter the gel, whereas linear viral DNA (Lin.) enters the gel and forms a discrete band. (b) Agarose gel mapping the double-strand break introduced by Bsu36I. Lane 1, DNA size standards from a HindIII digest of lambda DNA (S); lane 2, BglII digest of untreated AcRP6-SC viral DNA (U); lane 3, BglII digest of AcRP6-SC viral DNA previously digested with Bsu36I (sample D from (a)). Bsu36I digestion converts the 15 kbp BglII C fragment (solid triangle) into two fragments of 9.2 kbp and 5.8 kbp (open triangles). Sizes of DNA standards are given in kbp.

Table 1. Infectivity of linear and circular AcRP6-SC DNA.

Viral DNA	Yield of virus+ (10^4 p.f.u./μg DNA)	
	Median	Range
Circular AcRP6-SC	25	3.7–44
Linear AcRP6-SC	1.6	0.04–3.4

\+ median and range from 5 experiments.

assayed in this way, linear viral DNA had only about 1/150th the infectivity of circular DNA (Table 2, compare the total yields for no treatment and mock digested with that for digested DNA).

Recombination between linear viral DNA and a plasmid transfer vector

To assay recombination between viral and plasmid DNAs we used a polyhedrin based transfer vector in which the *lac*Z gene from *Escherichia coli* was coupled to the polyhedrin promoter (pAcRP23-*lac*Z; 19). Viruses that acquire the *lac*Z gene by recombination with this transfer vector produce plaques that contain β-galactosidase. Such plaques stain blue when incubated with X-gal (27) and can easily be distinguished from plaques of the parental virus which remain white. Initial experiments showed that when linear AcRP6-SC DNA was co-transfected with pAcRP23-*lac*Z DNA a higher fraction of the progeny viruses were recombinant than when the viral DNA was circular: for linear DNA the fraction of viruses that were recombinant ranged from 6% to 32% with a median of 19%, whereas for circular DNA this fraction ranged from 0.8% to 2.6% with a median of 2.0% (Table 2, and data not shown). We then compared a number of variations on linearization in order to find which treatment yielded the highest fraction of recombinant viruses (Table 2). Linearizing the viral DNA by cutting at the unique Bsu36I site increased the fraction of progeny viruses that were recombinant by about 10-fold. Neither dephosphorylation of the ends of the linear DNA (to prevent religation), nor heating of

the DNA immediately prior to co-transfection (to ensure that the sticky-ends were not annealed), made a significant difference compared to simple linearization. However, a 6-fold over-digestion of the AcRP6-SC DNA did increase the fraction of recombinant viruses by another 2-fold. By comparing the virus yields, it is apparent that the increase in the proportion of recombinant viruses on linearization of the viral DNA was the result of a large reduction, approximately 150-fold, in the total yield of virus but a much smaller reduction, approximately 12-fold, in the yield of recombinant viruses.

The effect of introducing a double-strand break at different locations in the AcMNPV genome

Having shown that linearization of AcMNPV DNA in the polyhedrin region increased the proportion of viruses that had recombined with a polyhedrin based transfer vector, we wished to determine whether this effect was specific for the polyhedrin locus or whether the introduction of double-strand breaks at other locations in the AcMNPV genome would have a similar effect. We therefore examined recombination between AcMNPV DNA linearized at the p10 locus and a p10 based transfer vector. The source of viral DNA for these experiments was AcUW1-*lacZ*, an AcMNPV derivative in which part of the p10 gene is replaced by the *E. coli lacZ* gene (16). The *lacZ* gene contains a recognition site for Bsu36I (28) therefore digestion with Bsu36I linearizes AcUW1-*lacZ* viral DNA at the p10 locus. The transfer vector we used in this study was pAcUW1-NA, a p10 based vector into which the neuraminidase gene of influenza virus had been inserted so as to be expressed from the p10 promoter (16; A.Cartwright, personal communication). Viruses that have recombined with this transfer vector can be distinguished from parental viruses using a simple colourimetric assay for neuraminidase activity (25). In experiments using this combination of viral DNA and transfer vector, the fraction of recombinants amongst the progeny viruses from co-transfections with linear DNA was approximately 10-fold higher than the fraction obtained from co-transfections with the circular form (Table 3). Linearization of AcMNPV DNA at the p10 locus thus produced a similar increase in the proportion of viruses that are recombinant to that resulting from linearization at the polyhedrin locus.

In these experiments, we could evaluate the contribution of undigested viral DNA to the non-recombinant background because the parental virus produces plaques which stain blue with X-gal. As expected, more than 95% of the progeny of co-transfections with circular viral DNA produced blue plaques, however, only about 20% of the viruses produced from co-transfections with linear viral DNA made blue plaques (data not shown). Thus, for co-transfections with linear DNA about 20% of the progeny viruses were recombinant, another 20% were parental and the remaining 60% were non-recombinant but no longer expressed a functional β-galactosidase. At most, therefore, undigested viral DNA only accounted for about 1/4 of the non-recombinant viruses produced in these experiments.

DISCUSSION

By constructing an AcMNPV derivative possessing a unique restriction site, we have been able to compare the infectivity and recombinogenic activity of circular and linear forms of the viral DNA. Linearized AcMNPV DNA was only 1/15th as infectious as the circular form when transfected into *S. frugiperda* cells using the calcium phosphate precipitation method (Table 1). In the presence of a 5-fold excess of plasmid DNA, the relative infectivity of linear DNA fell to approximately 1/150th that of circular DNA (Table 2). This reduced infectivity of linear AcMNPV DNA in *Spodoptera* cells parallels the reduction in transformation of *E. coli* by linearized plasmid DNAs (29).

Table 2. Effect of various treatments to AcRP6-SC DNA on recombination with a transfer vector.

Treatment	Yield of virus+ (10^4p.f.u./μg viral DNA) Total	Recombinant	Fraction of viruses that are recombinant* Median	Range
None	1000	8.5	0.9%	(0.8–1.4)
Mock Digested	260	3.4	1.3%	(0.8–2.3)
Digested with Bsu36I	3.5§	0.39§	14%§	(9.7–18)§
Digested + Dephosphorylated	1.2	0.28	21%	(8.3–23)
Digested + Heated	5.3	0.50	16%	(9.1–18)
6-fold Over-digested	0.21	0.04	37%	(16–39)

+ AcRP6-SC DNA was co-transfected with pAcRP23-*lacZ* and after 2 days the progeny viruses were titred. Plaques that stained blue with X-gal were scored as recombinant; median of 3 experiments.
* yield of blue plaques÷total yield; median and range of 3 experiments.
§average or range from 2 experiments.

Table 3. Recombination between a p10 based transfer vector and AcUW1-*lacZ* DNA linearized at the p10 locus.

Viral DNA	Yield of virus+ (10^5 p.f.u./μg viral DNA)	Fraction of viruses that are recombinant*
Circular AcUW1-*lacZ*	1.2	2.4%
Linear AcUW1-*lacZ*	0.11	22%

+ AcUW1-*lacZ* DNA was co-transfected with pAcUW1-NA and after 2 days the progeny viruses were titred; average of 2 experiments.
* plaques were scored as recombinant if they produced a pink colour in the neuraminidase assay; average of 2 experiments.

In a previous study, Kelly and Wang reported that linear DNA from *Trichoplusia ni* MNPV was not infectious (14). The difference between their findings and those reported here is likely to be due to the different methods used to prepare the linear viral DNA. Digestion of the viral DNA from an AcMNPV derivative possessing a unique restriction endonuclease site introduces one double-strand break into an otherwise intact molecule. Furthermore, breaks were introduced into regions of the viral genome which are known to be nonessential for growth of the virus in cell culture (23, 30, 31), therefore, even imprecise repair at these sites could yield a viable virus. In contrast, Bud and Kelly (26) and Kelly and Wang (14) employed crude methods to linearize viral DNA, i.e. controlled shearing, digestion with S1-nuclease or heat treatment of polyhedra. The infectivity of the linear DNA produced by these methods may have been reduced because the location of the break was not controlled and because the DNA may have suffered more damage than just one double-strand break.

Even though linear AcMNPV DNA was much less infectious in *S. frugiperda* cells than the circular form, it was still able to recombine with an appropriate plasmid after co-transfection. The low infectivity of linear viral DNA resulted in a great reduction in the background of non-recombinant viruses produced from a co-transfection, however, only a relatively small reduction in the yield of recombinant viruses was observed. Consequently, a high proportion of the progeny viruses, typically 10% to 25%, were recombinant. This represented a 10-fold higher fraction of recombinants than was obtained with circular DNA. A similar effect was seen for recombination with either a polyhedrin-based or a p10-based transfer vector if the AcMNPV DNA was linearized at the corresponding locus. The high proportion of recombinants following transfection of *S. frugiperda* cells with linear viral DNA was due to a reduction in the background of parental viruses rather than to an increase in the absolute number of recombinants obtained. This contrasts to the observation that linear DNA molecules are more recombinogenic than circular DNAs when transformed into yeast or mammalian cells (8, 9).

One possible explanation for the low infectivity of linear AcMNPV DNA is that only circular viral DNA can function as a substrate for replication. We favour this hypothesis because it seems unlikely that a virus whose native DNA is circular would encode the specialised machinery necessary to replicate linear DNA. In addition, this hypothesis can explain the high proportion of recombinant viruses obtained with linear viral DNA. Recombination between the viral sequences on either side of a double-strand break with homologous sequences on a transfer vector will restore the circularity of the viral DNA thereby enabling it to replicate (Figure 3). If a fragment of foreign DNA has been inserted between the homologous sequences on the transfer vector, then the recombination events that rescue the linear viral DNA will transfer the insert to the viral genome (Figure 3). This model predicts that an increase in the proportion of viruses that are recombinant should be observed for linearization at any locus on the baculovirus genome provided that the transfer vector carries viral DNA homologous to sequences on both sides of the break. We have shown such an increase for linearization at two different loci, polyhedrin and p10, although we have not tested the homology dependence of this effect.

Assuming that linear viral DNA is not able to replicate, the background of non-recombinant plaques in co-transfections with linear viral DNA and the residual infectivity of linearized DNA could come from two sources: (i) input viral DNA that had not suffered a double-strand break, and (ii) recircularization of linear viral DNA by processes other than recombination with homologous sequences on a transfer vector, e.g. ligation of the ends or illegitimate recombination between viral sequences on either side of the break. For co-transfections involving linear AcUW1-*lacZ* DNA, undigested viral DNA accounted for less than 1/4 of the non-recombinant progeny viruses; the majority of non-recombinant viruses no longer expressed functional β-galactosidase and appear to have been generated by processes that result in the disruption of DNA sequences around the double-strand break. Insect cells may thus process linear DNA in a similar manner to mammalian cells in which end-joining reactions are known to be very efficient but frequently imprecise (32).

One of the most laborious step in the construction of baculovirus expression vectors is the identification of a virus that has incorporated foreign DNA by recombining with a transfer vector. Our finding that linearization of the baculovirus DNA increases the proportion of viruses that have recombined with a transfer vector by about 10-fold should greatly facilitate the screening process. Linearization of the viral DNA at either the polyhedrin or p10 loci, as appropriate, could be used in conjunction with any of the AcMNPV transfer vectors currently in use to reduce the number of plaques that need to be screened by about 10-fold. Recently, several transfer vectors have been developed which facilitate the identification of recombinant viruses by placing a reporter gene adjacent to the foreign gene (16, 33–35). However, the use of linearized viral DNA to derive a recombinant virus has several advantages over the use of these vectors: (i) the size of the transfer vector can be kept to a minimum because no reporter gene is required, (ii) there will not be any competition between the gene of interest and the reporter gene for transcription or translation machinery, and (iii) there will not be any unwanted reporter protein to complicate purification of the desired product.

Although our experiments are limited to AcMNPV, we expect that linearization of the viral DNA from other baculoviruses, e.g. *Bombyx mori* MNPV and *Heliothis zea* SNPV, would facilitate the isolation of derivatives of these viruses that had acquired

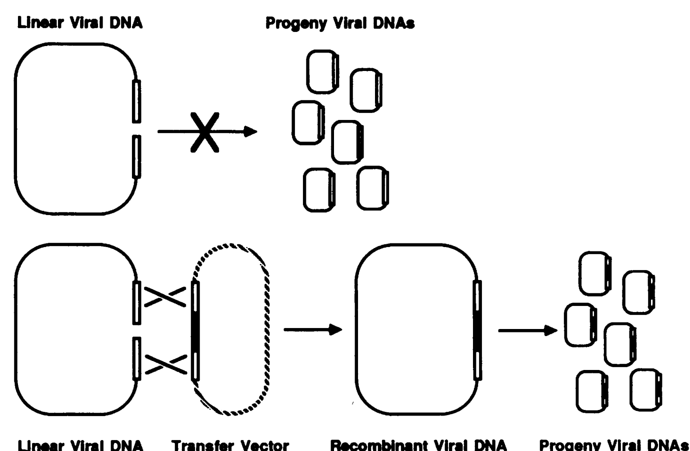

Figure 3. Rescue of linear viral DNA by recombination with a transfer vector. Upper panel: linear DNA cannot replicate because the replication apparatus of AcMNPV is designed to work on the native viral DNA which is circular. Lower panel: the circularity of the viral DNA can be restored by recombination with a transfer vector carrying DNA homologous to the viral sequences on either side of the break. A double crossover generates a recombinant viral DNA molecule which, being circular, is competent for replication.

modified sequences through recombination with the cognate transfer vector.

ACKNOWLEDGEMENTS

The authors thank Alison Cartwright and Ulrike Weyer for providing plasmids and viruses, Vince Emery for synthesis of the oligonucleotide and Chis Hatton for photography. We are also grateful to David Bishop for his comments on the manuscript.

REFERENCES

1. Luckow,V.A. and Summers,M.D. (1988) *Bio/Technology*, **6**, 47–55.
2. Miller,L.K. (1988) *Ann. Rev. Microbiol.*, **42**, 177–199.
3. Maeda,S. (1989) *Ann. Rev. Entomol.*, **34**, 351–372.
4. Fraser,M.J. (1989) *In Vitro Cell. Devel. Biol.*, **25**, 225–235.
5. Possee,R.D., Weyer,U. and King,L.A. (1990) In Griffiths,P., Dimmock,N.J. and Modeley,C.R. (eds.), Control of Virus Diseases. Cambridge University Press, pp.53–76.
6. Smith,G.E., Summers,M.D. and Fraser,M.J. (1983) *Mol. Cell. Biol.*, **3**, 2156–2165.
7. Miller,D.W., Safer,P. and Miller,L.K. (1986) In Setlow,J.K. and Hollaender,A. (eds.), Genetic Engineering. Plenum, New York, Vol. 8, pp. 277–298.
8. Orr-Weaver,T.L., Szostak,J.W. and Rothstein,R.J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6354–6358.
9. Bollag,R.J., Waldman,A.S. and Liskay,R.M. (1989) *Ann. Rev. Genet.*, **23**, 199–225.
10. Orr-Weaver,T.L., Szostak,J.W. and Rothstein,R.J. (1983) *Meth. Enzymol.*, **101**, 228–245.
11. Miller,L.K. (1981) *J. Virol.*, **39**, 973–976.
12. Gordon,J.D. and Carstens,E.B. (1984) *Virology*, **138**, 69–81.
13. Croizier,G., Croizier,L., Quiot,J.M. and Lereclus,D. (1988) *J. gen. Virol.*, **69**, 177–185.
14. Kelly,D.C. and Wang,X. (1981) *Ann. Virol. (Inst. Pasteur)*, **132E**, 247–259.
15. Possee,R.D. (1986) *Virus Res.*, **5**, 43–59.
16. Weyer,U., Knight,S. and Possee,R.D. (1990) *J. gen. Virol.*, **71**, 1525–1534.
17. Vaughn,J.L., Goodwin,R.H., Tompkins,G.J. and McCawley,P. (1977) *In vitro*, **13**, 213–217.
18. Matsuura,Y., Possee,R.D., Overton,H.A. and Bishop,D.H.L. (1987) *J. gen. Virol.*, **68**, 1233–1250.
19. Possee,R.D. and Howard,S.C. (1987) *Nucleic Acids Res.*, **15**, 10233–10248.
20. Bentley,D.R. and Brownlee,G.G. (1982) *Nucleic Acid Res.*, **10**, 5033–5042.
21. Maxam,A.M. and Gilbert,W. (1980) *Meth. Enzymol.*, **65**, 499–560.
22. Summers,M.D. and Smith,G.E. (1987) A manual of methods for baculovirus vectors and insect cell culture procedures. Texas Agricultural Experiment Station Bulletin No. 1555.
23. Smith,G.E., Fraser,M.J. and Summers,M.D. (1983) *J. Virol.*, **46**, 584–593.
24. Brown,M. and Faulkner,P. (1977) *J. gen. Virol.*, **36**, 361–364.
25. Barrett,T. and Inglis,S.C. (1985) In Mahy,B.W.J. (ed.), Virology: a practical approach. IRL Press, Oxford, pp. 119–150.
26. Bud,H.M. and Kelly,D.C. (1980) *Microbiologica*, **3**, 103–108.
27. Pennock,G.D., Shoemaker,C. and Miller,L.K. (1984) *Mol. Cell. Biol.*, **4**, 399–406.
28. Kalnins,A., Otto,K., Ruether,U. and Mueller-Hill,B. (1983) *EMBO J.*, **2**, 593–597.
29. Conley,E.C. and Saunders,J.R. (1984) *Mol. Gen. Genet.*, **194**, 211–218.
30. Vlak,J.M., Klinkenberg,F.A., Zaal,K.J.M.,Usmany,M., Klinge-Roode,E.C., Geervliet,J.B.F., Roosien,J. and Van Lent,J.W.M. (1988) *J. gen. Virol.*, **69**, 765–776.
31. Williams,G.V., Rohel,D.Z., Kuzio,J. and Faulkner,P. (1989) *J. gen. Virol.*, **70**, 187–202.
32. Wake,C.T., Gudewicz,T., Porter,T., White,A. and Wilson,J.H. (1984) *Mol. Cell. Biol.*, **4**, 387–398.
33. Vialard,J., Lalumiere,M., Vernet,T., Briedis,D., Alkhatib,G., Henning,D., Levin,D. and Richardson, C. (1990) *J. Virol.*, **64**, 37–50.
34. Vlak,J.M., Schouten,A., Usmany,M., Belsham,G.J., Klinge-roode,E.C., Maule,A.J., van Lent, J.W.M. and Zuidema,D. (1990) *Virology*, (in press).
35. Zuidema,D., Schouten,A., Usany,M., Maule,A.J., Belsham,G.J., Roosien,J., Klinge-Roode,E.C., van Lent,J.W.M. and Vlak,J.M. (1990) *J. gen. Virol.*, (in press).
36. Hooft van Iddekinge,B.J.L., Smith,G.E. and Summers,M.D. (1983) *Virology*, **131**, 561–565.
37. Howard,S.C., Ayres,M.D. and Possee,R.D. (1986) *Virus Res.*, **5**, 109–119.
38. Gearing,K.L. and Possee,R.D. (1990) *J. gen. Virol.*, **71**, 251–262.