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Packaging recombinant DNA molecules into bacteriophage particles *in vitro*

(bacteriophage λ morphogenesis/infectious DNA)

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ABSTRACT **Recombinant phage genomes made in reactions with purified enzymes may be recovered directly by packaging into phage heads *in vitro*. The process is efficient and nonselective and offers containment in initial stages of handling recombinant DNA. Ligase [poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase (AMP-forming), EC 6.5.1.1] reaction products can recombine with endogenous phage DNA during packaging, but UV-irradiation eliminates the biological activity of the endogenous DNA.**

Hybrid molecules may now be constructed *in vitro* between DNA fragments from various sources and bacterial plasmids or bacteriophage λ DNA (1–8). Since the plasmids and phage DNA are capable of autonomous replication, the recombinant molecules enable DNA fragments to be cloned in bacterial cells. In addition to autonomous replication, the principal requirements of the receptor DNA molecules are that they must be able to infect competent bacteria, that they contain one or a very limited number of targets for the restriction enzyme, that insertion of the new DNA fragment does not destroy a gene essential for the function of the plasmid or phage, and that cells carrying the plasmid or phage can be readily selected and the recombinant plasmids or phage distinguished easily from their parents (9–11). For phage λ DNA there is a further constraint in that only molecules in a certain size range can be packaged into a viable virion; some of the DNA must therefore be deleted from inessential regions of the phage genome in order to provide space for the new fragments and the recombinant molecule must have a length between about 75 and 105% of wild-type λ DNA (12). The joining of fragments of vector and donor DNA in reactions with polynucleotide ligase [poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase (AMP-forming), EC 6.5.1.1] usually yields a complex range of products which in practice are seldom fractionated. Instead, the mixtures are used directly to transform (13), or transfect (14), cultures of competent bacterial cells, thus allowing the cell to selectively propagate only those DNA molecules that are viable in this specific host strain.

Yields of transformed cells, or phage particles, from these procedures are variable. With intact DNA from lambdoid phages the yield of phage particles per μg in transfection experiments with *E. coli* is usually between 10^4 and 10^6; the average represents a recovery of less than 1 in 10^5 of the DNA molecules. With DNA preparations that have been restricted and the fragments rejoined by treatment with polynucleotide ligase, the yield of phage per μg of DNA in the reaction mixture is usually at least an order of magnitude lower than this.

Other procedures are available for conversion of DNA molecules into phage particles. Of these, the packaging of DNA into viable phage particles *in vitro* with extracts of appropriately lysogenized and induced cells gives recoveries as high as 1 in 10^3 of the DNA molecules.

The principle of *in vitro* packaging is the following: in the presence of high concentrations of the phage head precursor, or prehead, and the packaging proteins, which are the products of genes *A*, *Nu1*, *D*, and *FI* (15), λ DNA is packaged (16, 17). The full heads are then matured (*in vitro*) into plaque-forming units (PFU) in the presence of proteins from genes *W* and *FII* and phage tails (18). Practically, *in vitro* packaging is most efficiently performed in a very concentrated mixture of two induced lysogens, one of which is genetically blocked at the prehead stage (by an *amber* mutation in, e.g., gene *D*) and therefore accumulates these precursors, while the other is inhibited from forming capsids by an *amber* mutation in the gene, *E*, for the main capsid protein. These two lysates provide all the necessary components to convert λ DNA into a plaque-forming particle. Endogenous concatemeric phage DNA present in these lysates is packaged and cleaved (17, 19), but if mature linear DNA from another lambdoid phage is added to the reaction mixture, this also becomes packaged (16). It has been reported that circular, monomeric λ DNA, however, cannot be made infective by either transfection (20) or *in vitro* packaging (16, 19). In this communication we describe the application of this packaging system for making *in vitro* recombinant DNA infectious.

MATERIALS AND METHODS

Description of Vectors. The phage vector 540 is described in ref. 21; vectors 590, 598, 607, 641, 705, and 728 are described in ref. 11. The genotypes of the remainder are as follows: 459, λ (*srI*λ1–2) ∇*c*I857 *nin*5; 461, λ*b*538 *srI*λ3 *nin*5; 596, λ [(*srI*λ1–3)∇ (*supE*$^+$)] *sus*γ *c*I857 *nin*5; 616, λ*plac*5 *att*$^+$ *imm*21 *nin*5; 646, λ*plac*5 *srI-lac* srIλ3 *N*am *c*Its *nin*5; 711, λ(*srI*λ1–2)∇ *shn*λ3 (*trpE*$^+$) (*att-red*)∇ *sus*γ *c*V (KH54) *nin*5; and 722, λ(*srI*λ1–2)∇ *shn*λ3 (*supF*$^+$) (*att-xis*)∇ *red*$^+$ *imm*21 *nin*5. (The abbreviations used for restriction enzymes and their targets in phage genomes are given in refs. 11, 21, 22, and 23.)

Restriction and Ligation Reactions. Restriction enzyme digests were normally carried out with about 2 μg of DNA in 50 μl of a solution containing 10 mM Tris·HCl (pH 7.5), 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 50 mM NaCl for the *Hin*dIII enzyme, but 100 mM NaCl for the *Eco*RI enzyme; solutions were incubated at 37° for 1–2 hr, heated at 70° for 5 min, and kept at 0°. The necessary quantity of the restriction enzyme was determined in trial digests of λ$^+$ DNA that were analyzed by electrophoresis in 1% agarose gels (7). The restriction of the vector DNA samples was assessed from the reduction in transfection yield before proceeding with the addition of donor DNA fragments (restriction enzyme digests of

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Abbreviation: PFU, plaque-forming units.

Table 1. Yields of phage (per μg of vector DNA) in transfection and packaging experiments *in vitro*

Exp. no.	Phage DNA (vector)	Restriction targets	Before restriction		After restriction + donor DNA + ligation	
			Transfection	Packaging	Transfection	Packaging
1	461	1 *Eco*RI	2.5×10^4	8×10^6		
				11×10^6 *		
			2.9×10^4	6.5×10^5		
				2.5×10^6 *		
	607	1 *Eco*RI	4.6×10^4	1×10^7	4×10^3	1.5×10^6
				3.3×10^6 *		1.0×10^6
			5.1×10^4	1.7×10^6	4×10^2	7.6×10^4
				5×10^6 *		7.2×10^3 †
	λcI857Sam7	5 *Eco*RI	6.2×10^4	1.3×10^7		
				1.0×10^7 *		
2	461	1 *Eco*RI	2.9×10^4			
	641	1 *Eco*RI	1.2×10^5		3.7×10^3	8.2×10^4
	646	2 *Eco*RI	1.2×10^5		6×10^4	6.6×10^5
	540	1 *Hin*dIII	1.1×10^5		1.9×10^4	1.3×10^6
	590	1 *Hin*dIII	1.5×10^5		5×10^4	1.5×10^6
	598	1 *Hin*dIII	3.4×10^4		4.2×10^4	8.0×10^5
	705	2 *Hin*dIII	5.3×10^4		5.6×10^3	1.6×10^5
	711	2 *Hin*dIII	7.8×10^4		6.8×10^3	3.2×10^4
3‡	596	2 *Eco*RI	$0.15–5 \times 10^5$	$1.7–8.0 \times 10^7$	0.8×10^3	$0.1–0.5 \times 10^5$
	722	2 *Hin*dIII	$0.09–1.8 \times 10^5$	$0.1–6.0 \times 10^7$	$0.1–9.0 \times 10^3$	$0.1–7.6 \times 10^5$
	598	1 *Hin*dIII	1×10^5	4.4×10^5	3×10^3	1.7×10^5

The packaging in Exps. 2 and 3 was performed with UV-irradiated cells (see legend to Fig. 1 for procedure). Phage were plated on *E. coli* C600. The yields of phage shown for the restriction and ligation experiments are total phage; the recombinants were recognized in the various experiments by their clear plaque morphology [vectors 607, 641, 590, and 598 (9, 11)], by complementation of an auxotrophic host strain, or by their ability to plate on a *pel*$^-$ host strain since this is dependent upon the length of the phage genome (ref. 31; and W. Arber and H. Jütte, personal communication).

* Duplicated experiments were done simultaneously with independently prepared batches of cells for the packaging mixtures.

† An experiment done simultaneously with yeast DNA as donor gave 500 PFU/μg of DNA in transfection, but 4.7×10^4 and 1.4×10^4 PFU/μg of DNA in duplicate packaging experiments *in vitro*.

‡ This group of experiments was carried out by the participants in the EMBO course referred to in the *text*. The combination of donor and receptor DNA was different in each experiment: the donors were DNA from different *E. coli* strains, a λ*trp* transducing phage, or plasmids carrying all or part of the *E. coli trp* operon. The latter were kindly provided by V. Hershfield and D. R. Helinski. The results are given as the range of yields from three independent experiments with the 596 vector and four independent experiments with the 722 vector.

E. coli DNA) and reaction with T4 polynucleotide ligase (23, 24). The solutions were diluted to a DNA concentration (vector molecules) of 5–10 μg/ml and incubated for 3 hr at 10° followed by at least 24 hr at 0° with polynucleotide ligase from *E. coli* infected with phage T4 (Miles Laboratories Ltd., Stoke Poges, Bucks, U.K.), (0.1 unit/ml or 2 μl of the concentrated enzyme preparation per ml of reaction mixture; the optimum concentration was determined experimentally for each batch of enzyme by measuring the restoration of transfection yield of a restriction enzyme digest of a phage DNA with a single target for *Eco*RI) in a mixture containing 66 mM Tris·HCl (pH 7.5), 1 mM EDTA, 10 mM $MgCl_2$, 5 mM dithiothreitol or 10 mM 2-mercaptoethanol, 40 mM NaCl, and 0.1 mM ATP.

Transfection. *E. coli* 803 *supE*$^+$ *supF*$^+$ $r^-_K m^-_K$ or $r^-_K m^+_K$ was grown in a poor medium and starved in 0.1 M $CaCl_2$ solution (14) or, most often, grown in L broth, washed in 0.01 M $MgSO_4$ solution, and starved in 0.1 M $CaCl_2$ solution (13). DNA was usually added to the competent cells to give a final concentration of about 1 μg/ml and diluted to 1/10 its original concentration before plating in the case of intact phage DNA; it was added at a concentration of about 0.5 μg/ml and plated undiluted in the case of restricted DNA solutions. Mixtures from ligase reactions were diluted to a DNA concentration of about 0.5 μg/ml with 150 mM NaCl/15 mM sodium citrate before they were mixed with the competent cells (two volumes) and these also were plated undiluted.

Packaging. Genomes were packaged *in vitro* as described (17) with some slight modifications: single colonies of the strains W3101 (λ*imm*434*c*It*s*Eam4Sam7) and W3101 (λ*imm*434*c*-It*s*Dam15Sam7) were streaked out on an LA plate (17) and grown overnight at 32°. The cells were suspended in warmed LB medium (17) at an OD_{600} of not more than 0.15 and incubated with shaking until an OD_{600} of 0.3 was reached. Induction was effected by incubation of the cultures at 45° for 15 min, standing. Thereafter they were transferred to 37° and incubated for 3 additional hours with vigorous aeration. (A small sample of each culture, which is lysis-inhibited as a result of the mutation in gene *S*, should be checked for induction; upon addition of a drop of $CHCl_3$, the culture should clear.) The two cultures were then mixed, centrifuged at 5000 rpm for 10 min, and resuspended in M9A medium (25), but without casein hydrolysate, at an OD_{600} of 0.3. For experiments without UV treatment (Tables 3 and 4) the cells were concentrated about 500-fold in complementation buffer (40 mM Tris·HCl, pH 8.0/10 mM NaN_3/10 mM spermidine/10 mM putrescine/0.1% 2-mercaptoethanol/7% dimethyl sulfoxide). UV-irradiated cells (Fig. 1) were concentrated in the same buffer. [At this stage the cells can be prepared for storage or used directly. Samples of 10–20 μl are distributed in polypropylene or nylon centrifuge tubes (1 ml), frozen rapidly in liquid N_2, and stored at −57° or below. When needed, a sample is then transferred in liquid N_2 and put on ice; ATP is added to a final concentration of 1.5 mM. If cells are to be used directly, ATP can be added before distribution of samples.] After freezing in liquid N_2 and thawing in ice (which takes 2–4 min), the DNA to be packaged (0.01–0.2 μg) was added and carefully mixed with the concentrate *immediately after thawing*. The mixture was centrifuged to the bottom of the tube by spinning for a few seconds to avoid unnecessary evaporation during the subsequent 30- to 60-min incubation at 37°. At the end of this incubation period 2 μl of

a DNase solution (100 μg/ml) was added; after further incubation at 37° for 10 min, 0.5 ml of SMC (17) and a drop of $CHCl_3$ were added. Phage obtained by this procedure can, when freed from debris, be stored as any phage lysate.

Containment Conditions. EK1 bacterial and bacteriophage strains were used. Physical containment was P1 except when yeast DNA was involved, where it was P2.

RESULTS AND DISCUSSION

Efficiency of Packaging *In Vitro*. Table 1 summarizes a series of experiments in which DNAs from a selection of *Eco*RI and *Hin*dIII vectors as well as recombinants made with *E. coli* DNA by restriction and ligation reactions were compared for their transfection and packaging efficiencies *in vitro*. Some of the data were collected by the participants of an EMBO (European Molecular Biology Organization) course on "DNA restriction endonucleases: Reactions and applications" (Basel, 1976), and therefore should be fairly representative of the efficiencies, and the variations thereof, obtainable. Generally, packaging of vector DNA *in vitro* yielded plaques with an efficiency about a 100-fold higher than transfection. Restricted and ligated DNa was packaged with an efficiency 10- to 100-fold higher than that from transfection.

Storage of Cells for Packaging. Since gentle lysis of the packaging cells is accomplished by freezing in liquid nitrogen and thawing, the possibility of storing the induced lysogens as a concentrated frozen mixture was investigated because it would obviously be a great convenience to make a large batch of cells and remove small samples as required. The competence of such a mixture for packaging *in vitro* remained at the original level for at least 14 months, the longest time tested, on storage at −57°. Survival of packaging activity upon storage at −24°, however, was less satisfactory.

Packaging Is Nonselective. Lambdoid phages with different genotypes vary appreciably in their growth characteristics on a given host and further differences in efficiency of phage growth are frequently encountered when a given phage is grown on different host strains. Both host and phage recombination systems markedly affect the ease of handling a particular phage; those with chromosomes of abnormal size or with reiterated sequences are relatively unstable and therefore become replaced in a population by more stable derivatives. One would thus not expect all of the members of a population of recombinant DNA molecules made *in vitro* to be equally viable, so that in transfection experiments with a large mixed population of DNA molecules, there may well be selection against some recombinants and a variable yield of each member of the family of recombinants, some of which may be rapidly lost altogether (26). If single plaques are isolated after transfection and propagated separately, these arguments obviously do not apply, but some recombinants may have been lost already because they were unable to grow on the host strain used for transfection.

Packaging of the DNA molecules *in vitro* does not offer the same opportunities for selection of some of the recombinants at the expense of others or for selection of the parental molecules if these have higher viability than the recombinants. Since the DNA is merely a passive packaging substrate and only DNA sequences very close to the ends have to be specific (16), the characteristics of the DNA molecule being packaged should be of little consequence and the composition of the population of phages produced in the "lysate" should reflect the relative abundance of the various DNA molecules present.

Table 2 shows that within the normal range of genome length for viable lambdoid phages, there is little difference in yield of viable phages in the packaging of genomes *in vitro* with deletions of various size. Table 2 also shows that, as expected,

Table 2. Efficiency of packaging *in vitro* of λ DNA preparations having different genome lengths and *red* genotype

Exp. no.	Phage DNA (vector)	% λ⁺ DNA deleted	*red* genotype	Packaging efficiency, PFU × 10^{-7}/μg DNA
1	λcI857Sam7	0	+	1.3
	616	10	+	1.7
	459	16	+	1.2
	607	18	+	1.5
	461	20	−	0.78
2	λcI857Sam7	0	+	0.68
	596	6	−	0.32
	598	18	−	1.7
	607	18	+	0.44
	641	20	−	0.94
	728	20	−	1.0

Packaging *in vitro* was performed with UV-irradiated cells (see legend to Fig. 1 for procedure) and phage were plated on *E. coli* C600.

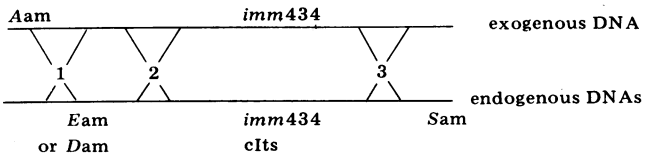
red⁻ phages do not appear to be at a disadvantage compared to *red*⁺ phages. More generally, the results of Tables 1 and 2 show little variation in yield attributable to phage genotype. In contrast with these results, Sternberg *et al.* (27) recently found that partially purified extracts from a different combination of cells preferentially packaged normal sized phage genomes and could thus be made selective for particular classes of recombinants. Also, in several experiments where the system described here was used with a mixture of a receptor genome (having a deletion of about 18%) and its *in vitro* recombinant, the recombinant was obtained in a noticeably higher yield (about 3-fold) whereas this was not the case with transfections (B. Klein, B. Hohn, and K. Murray, unpublished work). In any event *in vitro* packaging of a ligation reaction mixture gives a population of phages that has not been exposed to any of the selective pressures that accompany replication and growth of the phage within host cells. Moreover, since packaging is independent of replication, the recovery of independent DNA molecules out of a mixture is possible. The *in vitro* packaged phage can be kept indefinitely and scored by any selection method on various host strains whenever needed, but when the phage resulting from the packaging reactions are subsequently propagated they are, of course, subject to the normal factors encountered during growth within host cells. At the stage of *recovery* of the *in vitro* recombinant DNA molecules, however, the whole population is probably obtained without appreciable selection.

Recombination during Packaging. Several genetic markers were used to investigate recombination *in vitro* between the endogenous DNA and the added DNA at three different intervals along the λ genome. Table 3 shows that a low level of recombination in the packaging mixture was indeed detected and was more frequent in the central region of the chromosome, possibly due to the action of the phage integration enzyme, which functions *in vitro* (19, 28, 29).

One of the more useful λ vectors has the immunity region of phage 434 substituted for its own; its only *Eco*RI restriction target is located within the 434 *cI* gene (i.e., the gene for repressor) (9, 11). Insertion of a DNA fragment at this site inactivates the *cI* gene so that recombinant phages are distinguished from the vector because they form clear plaques. When this vector and donor (*E. coli*) DNA were restricted with *Eco*RI and the fragments were joined, recombination with endogenous DNA occurred during packaging. This recombination occurred at a variable efficiency that was sometimes high, but that de-

Table 3. Recombination of unrestricted exogenous DNA with λ DNA endogenous in the packaging mix

Indicator strain (*E. coli*)	*amber* mutations of λ suppressed	DNA packaged *in vitro*, PFU/μg	Frequency of recombination (× 10^4) in interval 1	2	3
C600 (*sup*E$^+$)	*A, D, E,* but not *S*	1.4×10^7, turbid plaques			
		3.2×10^3, clear plaques (~10 × higher than S^+ revertants of packaged endogenous DNA)			2.3
		5.6×10^4 *A*am$^+$ *D*am or *A*am$^+$ *E*am, turbid plaques		40	
W3101 (*sup*)^0	None	3.5×10^3, turbid plaques (7 × higher than *A*am$^+$ revertants)	2.5		



Packaging *in vitro* was performed with nonirradiated cells, as described in the legend to Table 1. λ*imm*434 *A*am32 DNA was used as exogenous DNA. When packaged, it produces turbid plaques on *E. coli* C600. Recombinants in interval 3 make a clear plaque on the same indicator at 37° due to the temperature-sensitive repressor. Recombinants in interval 2 plate on a *sup*^{0} (λ*A*am32) lysogen but not on a *sup*^{0} (λ*E*am4) or *sup*^{0} (λ*D*am 15) lysogen. Recombinants in interval 1 form turbid plaques of the *sup*^{0} indicator *E. coli* W3101.

creased with the period of incubation (at about 40°) of the ligation reaction mixture (Table 4). Moreover, there was a hot spot of recombination at an interval between immunity and gene *S* which gave *imm*434 *c*Its S^+ *E*am4 and *imm*434 *c*Its S^+ *D*am15 recombinants at a frequency two to three orders of magnitude above the *S*am7 to S^+ reversion rate observed in control complementation mixtures from which exogenous DNA was omitted. This recombination was not dependent upon the ligase (which normally is retained, but not inactivated, in the ligated sample) since heat inactivation prior to packaging *in vitro* did not reduce recombination with the endogenous phage DNA. The mechanism of this recombination is unknown, but possibly the ligated DNA molecules, or more probably large DNA fragments remaining after incomplete ligation or perhaps resulting from the action of a contaminating exonuclease, may initiate or enhance recombination with the endogenous phage DNA. The *Eco*RI target within *imm*434 is located 100 base pairs to the left of the O_R operator and therefore most likely to the right of the *ts* marker (V. Pirrotta, personal communication), which is consistent with this view.

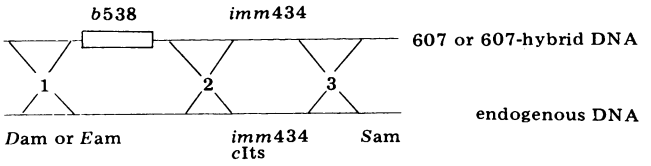
Packaging and Containment. Packaging *in vitro* avoids the necessity of cycling phage DNA molecules through living cells in order to recover them as phage particles. When used as the initial step in recovery of DNA molecules made in recombination research *in vitro*, it removes one stage at which viable phage could escape from the laboratory into the wider environment.

In addition to this degree of containment of the recombinant DNA, the final step in the packaging reactions *in vitro* includes treatment with chloroform, a procedure that has been recommended as a chemical means of containment (sterilization in this case) since it kills bacterial cells very effectively (30). However, any procedure that converts DNA or an *in vitro* recombinant thereof into plaque-forming phage should avoid or minimize the possibility for dissemination of a new and conceivably hazardous sequence via exchange with endogenous DNA in the system being used, be this *in vitro* packaging or transfection. The recombination observed between exogenous, unrestricted DNA, as well as the products of restriction and ligation reactions, and DNA endogenous in the *in vitro* packaging mixtures is therefore important in this context.

There are, in principle, two ways to eliminate recombination between the λ DNA added to the packaging extract and the *E. coli* or phage DNA that is already present in it: genetic elimination of all the known recombination systems from the lysogens used for the packaging mixtures, or elimination of the biological activity of the endogenous DNA. The latter course

Table 4. Effect of UV irradiation upon recombination of restricted and ligated vector DNA with λ DNA endogenous in the packaging mix

UV	Efficiency*	Interval†	% recombinants‡	Recombination in interval§ 1	2	1+2	2+3	3
			Exp. no. 1					
−	2.5×10^6	4	89					16/16
			Exp. no. 2					
−	3.3×10^4	1	27	3/30	5/30	4/30	3/30	15/30
−	1.3×10^4	5	7			4/35		31/35
−	1.7×10^5	18	<1					
			Exp. no. 3					
−	2.1×10^4	1	49	1/172		17/172		154/172
+	2.1×10^4	1	<0.0091					
−	3.4×10^5	3	5.2	1/23		1/23		21/23



Packaging *in vitro* was performed as described in the legend to Table 1. For Exps. 1 and 2 only nonirradiated cells were used; but Exp. 3 both unirradiated cells and cells irradiated for 30 min were used. Restriction of vector and donor (*E. coli*) DNA, and the ligation reactions were as described in the legend to Table 1. The ligated samples were kept at 4° for the number of days indicated, without inactivation of the ligase. The proportion of the recombinants between exogenous and endogenous DNA was calculated by comparing the plating properties of individual, purified plaques on *E. coli* C600 (*supE*$^+$) and a *sup*^{0} strain. Unrecombined 607 vector DNA or a ligation product thereof plates on both indicators. The majority of the recombinants (as detected by this plating procedure) had picked up an *amber E* or *D* mutation from the endogenous DNA. *S amber* mutations carried by a recombinant were not screened because their detection requires a *supF*$^+$ suppressor in the indicator strain, and this would have enabled phage containing the endogenous DNA to plate as well. Individual plaques, purified on *E. coli* C600, were tested for: *imm*434 (turbid plaques at both 32° and 40°); insertion of DNA fragment in *imm*434 [clear plaques at both temperatures (9, 11)]; *imm*434 *c*Its (turbid plaques at 32°, but clear ones at 40°); *b*538 deletion [inability to plate efficiently on GL1 *pel*21 W3101*supE*$^+$, provided by S. Emmons (25)]; and *D*am or *E*am (by their inability to plate on a *sup*^{0} host).

* PFU/μg of vector DNA in ligation reaction mixture.
† Days between ligation and packaging.
‡ % of total PFU plating on C600.
§ Fraction of total recombinants tested.

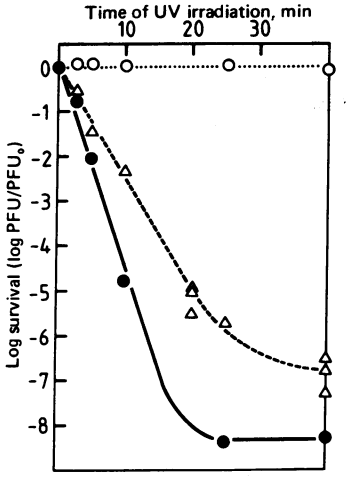


FIG. 1. Inactivation of bacteriophage λ and an *in vitro* packaging mixture by irradiation with UV light. In order to assess the required UV dose, λ*c*I857 *S*am7 phage and the packaging mixture prepared as described in the legend to Table 1 were each resuspended in M9 medium at an OD_{600} of 0.3 and distributed in empty sterile petri dishes in 10-ml portions. UV irradiation and all subsequent handling was performed in a darkroom, with the UV lamp (Westinghouse Sterilamp) as the only light source. At various time intervals after irradiation at a 50-cm distance, the phage titer was determined and the packaging cells were concentrated by centrifugation. Packaging *in vitro* was carried out as described in the *text*, using λ*c*1857 *S*am7 DNA as the exogenous DNA. Plating was on *Ymel*(λ) for phage that had packaged endogenous DNA and on *Ymel* (λ*imm*434) for phage containing the DNA provided exogenously (25). ●, Free phage; △, endogenous DNA packaged; ○, exogenous DNA packaged.

proved to be preferable because introduction of recombination deficiencies into host and prophage of the strains used for packaging reduced the efficiency of *in vitro* packaging by a factor of 2 to 5 (B. Hohn, unpublished work). Extensive irradiation of recombination-proficient packaging cells with UV light prior to use in the *in vitro* packaging reactions did not reduce their efficiency for packaging exogenous DNA, but greatly reduced the yield of endogenous DNA, either packaged or as free phage (Fig. 1). With packaging cells treated in this way no recombinants were detected during packaging of exogenous DNA (Table 4, Exp. 3).

These experiments show that the recombination during *in vitro* packaging can be controlled according to the requirements of a given experiment. With recombinant phage DNA made with a prokaryotic donor DNA, it may sometimes be of interest, or use, to retain this possibility; with donor DNA from higher eukaryotes, it would appear advisable to eliminate the opportunity for recombination as a precaution against possible incorporation of donor DNA sequences into the endogenous DNA from which they could eventually disseminate further. With this added precaution, *in vitro* packaging offers an efficient means for recovery of phage DNA molecules made by the methods currently used to construct heterologous recombinants *in vitro*, and at the same time provides for containment of the recombinants during the process.

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