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NOTES

Procaryotic Genomic DNA Inhibits Mammalian Cell Transformation

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Ltk$^-$ mouse cells were transformed to thymidine kinase prototrophy in the presence of carrier DNAs isolated from different organisms. Procaryotic genomic and phage DNA was consistently less effective as a carrier than was eucaryotic DNA. Mixing experiments indicate that DNA of procaryotic origin inhibits mammalian cell transformation.

The calcium phosphate ($CaPO_4$) DNA delivery system has been used to introduce a number of cellular and viral genes into appropriately marked cultured mammalian cells (9, 15, 16, 19). It has been observed that the transformation frequency when either viral or cloned DNA is used as donor material is significantly increased when exogenous DNA is included in the transformation as carrier (5, 6, 17). When transformations are conducted with cellular DNA, no carrier is needed because the cellular DNA itself acts as carrier.

Other than serving as a large nucleus for the formation of the proper $CaPO_4$-DNA precipitate (7), it is not clear what precise roles the carrier DNA has in facilitating transformation. Since it does not increase transformation efficiencies when the donor DNA is injected into nuclei (1), it may facilitate transport across the cytoplasm and nuclear membrane. Perucho et al. (10) have reported that in the recipient genome, DNA sequences which flank the selected marker originate from the carrier. This and other evidence suggest that donor DNAs are ligated to form large transgenomes before integration (11, 12). It has been postulated that carrier DNA may facilitate the maintenance of transgenomes before chromosomal integration by supplying sequences which act as origins for autogenous replication of these molecules (13).

We have used the transformation of thymidine kinase-deficient mouse cells (Ltk$^-$) with the cloned herpes simplex virus thymidine kinase (*tk*) gene as a model system (9) in evaluating the effects of using carrier DNAs from different biological sources. The source of donor *tk* was the plasmid pB12 (a gift of Paul Berg, Stanford University), which contains the herpes simplex virus *tk* gene inserted into the *Bam*HI site of pBR322 (4). Transformations were conducted by the method of Graham and van der Eb (6), with minor modifications (14). A total of 1 ng to 1 μg of pB12 DNA was used, so that 10 to 100 *tk*$^+$ transformants were obtained in HAT media. Carrier DNA was added at 20 μg/ml since results from this and other laboratories have determined this to be an optimal concentration (5). The results indicate a dramatic effect on the transformation efficiency when carrier DNA from different organisms was used (Table 1). Depending on the source, DNA from procaryotes was 2 to 3 orders of magnitude less effective as carrier than eucaryotic DNA.

We considered the possibility that eucaryotic carrier DNA contains sequences which are absent in procaryotic DNA and which allow for the maintenance or subsequent integration of the transgenome. λ DNA was observed to be as ineffective as genomic DNA from procaryotic organisms (Table 1). To determine whether this is due to the absence of particular eucaryotic sequences, we utilized DNA from a λ library containing random genomic inserts of the mouse AJ line (a gift from D. Mathus, Stanford University) as a carrier. One carrier consisted of DNA isolated from a random pool of approximately 200,000 different recombinant phages. In addition, DNAs isolated from three random, plaque-purified phage were each individually used as carrier. Recombinant λ DNA was used either alone or mixed with 5 or 10 μg of high-molecular-weight *Escherichia coli* LE392 or mammalian (Ltk$^-$) DNA to ensure the formation of consistent precipitates. The results obtained when these λ mouse recombinant DNAs were used as carriers are shown in Table 2, which indicates

TABLE 1. Tk transformation with different sources of carrier DNA

Carrier[a]	Expt	Colonies per μg of pB12 × 10^2 (mean ± SD)
Ltk⁻	1	383 ± 63
	2	120 ± 30
CF11-4	1	264 ± 76
HeLaS3	1	158 ± 84
	2	120 ± 40
Calf thymus	2	70 ± 20
Salmon sperm	2	60 ± 10
HB101	2	0.10 ± 0.1
LE392	1	0.61 ± 0.17
	2	0.10 ± 0.1
SB1117	1	0.35 ± 0.03
	2	0.30 ± 0.10
SB19	2	0.50 ± 0.10
λ-gt7	1	2.7 ± 2.1
pB12	1	0.15 ± 0.02

[a] DNA isolated from different biological sources was used as carrier in the transformation of Ltk⁻ cells to tk prototrophy. Ltk⁻ (mouse fibroblasts), CF11-4 (Chinese hamster) and HeLa S3 (human) DNA was isolated from cultured cells by phenol extractions as described previously (9). The calf thymus and salmon sperm DNA was obtained from Worthington Diagnostics. *E. coli* strains HB101 (*leuB6 recA16 proA2 lacZ4 thi-1 supE44 hsdR hsdM* Sm^r) and LE392 (*leuB6 thy thr-1*) and the *B. subtilis* strains SB19, a prototroph, and SB1117 (*trpC2 leuB*) were from the Stanford collection. DNA from protoplasts was isolated by phenol extractions followed by spooling of the ethanol precipitate. λ-gt 7 DNA (gift from Ron Davis) was isolated as described previously (3). The plasmid pB12 was obtained by the method of Clewell and Helinski (2) without amplification.

that there was no increase in the transformation efficiency when eucaryotic DNA was covalently linked to λ vectors compared with the use of the parental vector alone. DNA isolated from each of the plaque-purified recombinant phage, as well as the phage pool, was ineffective as carrier. We also observed in these experiments that, when Ltk⁻ DNA was included with the λ DNA in a 1:1 ratio, the transformation frequency was less than 10% that obtained with pure Ltk⁻ DNA as carrier. This suggests that the decrease in transformation efficiency is not because Ltk⁻ sequences are limiting, but rather that the λ DNA is inhibitory. pBR322 DNA has also been reported to have an inhibitory effect in transformation (16).

We wanted to determine whether the decrease in transformation efficiency obtained when either *E. coli* or *Bacillus subtilis* genomic DNA was used as carrier might also result from inhibition effects. This hypothesis was explored by competition experiments. In the first type of experiment, increasing amounts of *E. coli* LE392 DNA were mixed with either 5 or 10 μg of Ltk⁻ DNA, and these mixtures were used as carrier. In the presence of *E. coli* LE392 DNA, there was a dramatic decrease in the number of *tk*$^+$ transformants in each case (Fig. 1). Addition of 2 μg of *E. coli* LE392 to 10 μg of Ltk⁻ reduced transformation efficiencies by about 25 times. Reducing the concentration of Ltk⁻ DNA by 50% did not result in significantly fewer *tk*$^+$ clones, demonstrating again that, as in the λ recombinant experiments, Ltk⁻ sequences are not limiting.

Because the transformation frequency is dependent upon the total concentration of carrier DNA, a second type of mixing experiment was conducted with the final concentration of carrier constant while the ratio of bacterial to mammalian DNA was altered (Fig. 2). The reduced transformation efficiencies obtained in the presence of bacterial DNA was consistent with those

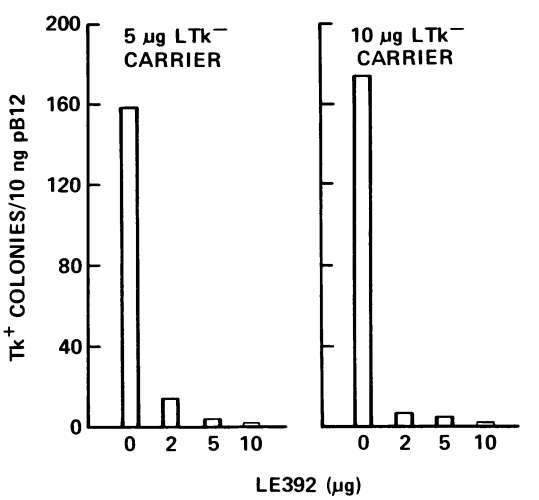

FIG. 1. *tk* transformation with mixed carrier DNA and a constant amount of Ltk⁻ DNA. Either 5 or 10 μg of high-molecular-weight Ltk⁻ DNA was mixed with 2, 5, and 10 μg *E. coli* LE392 DNA in a total volume of 0.5 ml. *Sal*I linearized pB12 (0.001 to 1 μg) was included as a selectable marker to identify transformants in HAT (selective medium containing hypoxanthine, aminopterin, and thymidine [20]) selection. The average number of *tk*$^+$ transformants for 5 plates is shown for each DNA mixture. There was a dramatic decrease in transformation efficiency when *E. coli* LE392 DNA was added.

TABLE 2. Use of λ-mouse recombinant DNAs as carrier[a]

Carrier (amt [μg])	tk$^+$ colonies per 0.1 μg of pB12 DNA (mean ± SD)			
	λ-AJC	λ-SP1	λ-SP2	λ-gt4
λ (10) + Ltk$^-$ (0)	6 ± 2	0 ± 1	2 ± 1	4 ± 2
λ (5) + Ltk$^-$ (5)	12 ± 6	22 ± 2	12 ± 2	ND
λ (5) + LE392 (5)	0 ± 2	4 ± 2	1 ± 1	ND
λ (0.5) + Ltk$^-$ (10)	186 ± 70	410 ± 12	214 ± 45	170 ± 26
λ (0.5) + LE392 (10)	0 ± 0	4 ± 1	0 ± 0	4 ± 2

[a] The cloning vector λ-gt4 containing random inserts of the mouse genome was used as carrier in tk transformations with pB12 DNA. λ-AJC is a mixed pool of recombinants containing approximately 200,000 phage. λ-SP1, λ-SP2, and λ-SP3 are single plaque isolates chosen at random from the library. The λ DNAs were also mixed with genomic DNA from Ltk$^-$ or *E. coli* LE392 with a final DNA concentration of 20 μg/ml in each case. Numbers shown are the average ± standard deviation of tk$^+$ colonies per 0.1 μg of pB12 DNA from three plates containing 5 × 10^5 Ltk$^-$ recipients.

found in previous experiments. When *E. coli* LE392 DNA constituted 30% of the carrier, there was a 20-fold decrease in transformation efficiency. These experiments indicate that, like pBR322 and λ DNA, bacterial genomic DNA inhibits mammalian cell transformation.

We have not yet determined a molecular mechanism for the inhibition of transformation by bacterial DNA. The inhibition was not due to contaminants in the bacterial DNA preparations being toxic to the mouse cells, since in nonselective media, the plating efficiencies of recipients exposed to different DNAs was similar (data not shown). Our experiments to evaluate the efficiency of DNA uptake when different carriers were used were inconclusive. Another possibility is that procaryotic DNA inhibits expression, replication, or recombination of the donor transgenome in the transformants. Of some bearing on the second possibility is the observation of Lusky and Botchan (8) that a 430-base-pair sequence on pBR322 inhibits replication of simian virus 40 pBR322 plasmids. We have observed the inhibition of transformation by bacterial DNA even when it is introduced unlinked to the selectable DNA. The replication or transcription of the *tk* gene may be inhibited after intracellular recombination with bacterial carrier DNA, but if recombination is random, a linearity between the amount of procaryotic DNA and the transformation efficiency would be expected. This linearity was not observed; the magnitude of inhibition is much greater than what would be expected from the *tk*$^+$-to-bacterial DNA ratio. Another hypothesis for the inhibitory effect is that bacterial DNA is often lethal when introduced into recipient cells. If the lethality of bacterial DNA were a function of the nature and location of integration, certain transformants might still contain a high copy number of bacterial donor genes (18, 20). A fourth hypothesis is that the bacterial DNA *tk* donor transgenome is preferentially eliminated from the recipients. Such a mechanism might be similar to restriction modification systems in bacteria.

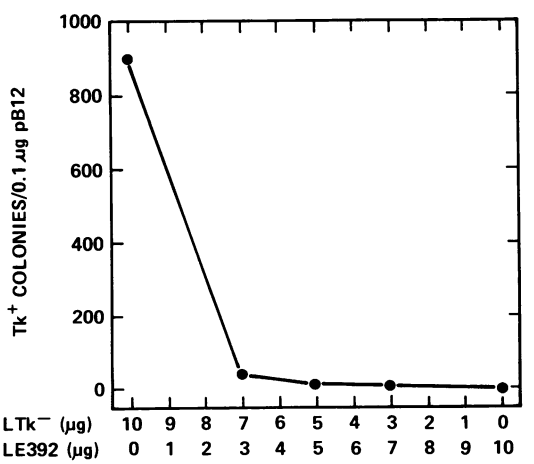

FIG. 2. *tk* transformation with mixed carrier DNA and a constant final DNA concentration. pB12 DNA was transformed into Ltk$^-$ cells with a mixture of Ltk$^-$ and *E. coli* LE392 DNA as carrier. Each of the DNAs were precipitated in 0.5-ml volumes for a final DNA concentration of 20 μg/ml. Transformation and selection was done as before. The average number of *tk*$^+$ transformants for five plates of 5 × 10^5 recipient cells is shown for each point.

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