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Cell Biology

Gene transfer to human cells: Transducing phage λ*plac* gene expression in GM_1-gangliosidosis fibroblasts

(enzyme-deficient cells/*Escherichia coli* β-galactosidase/protecting antibodies/ion-exchange chromatography)

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ABSTRACT **Genetic information from the bacterium *Escherichia coli* was transferred to human cells by means of the specialized transducing phage λ*plac* carrying the bacterial *z* gene for the enzyme β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23). As recipient cells, cultured skin fibroblasts from a patient with generalized gangliosidosis (GM_1-gangliosidosis Type I) characterized by a severe deficiency of β-galactosidase activity were used. The deficient human cells were incubated with the bacteriophage λ*plac* or λ*plac* DNA and β-galactosidase activity was measured in order to detect gene transfer and acceptance of the prokaryotic information in the mammalian system for transcription and translation. The expression of the phage genome in the deficient fibroblasts could be demonstrated by detection of higher β-galactosidase activity after incubation with phage λ*plac* in three out of 19 experiments and in four out of 16 experiments after treatment with λ*plac* DNA. λ*plac* DNA induced much higher enzyme activities than infective phage particles. Immunochemical and physicochemical assays could not distinguish the induced β-galactosidase activity from that of the *z*-gene product of *E. coli*.**

Evidence has been presented that genetic material of prokaryotic origin can be accepted by the eukaryotic machinery for transcription and translation (1–13). The expression of a bacterial-derived viral gene in human cells was described by Merril *et al.* (2, 3). They infected† galactose-1-phosphate uridyl transferase negative fibroblasts from a galactosemic patient with the *gal*-transducing bacteriophage λ*pgal*. Demonstration of phage-specific RNA and enzyme activity in the infected cells together with a longer survival on galactose medium with respect to uninfected cells was the major success of those experiments. Since then, bacterial genes have been successfully introduced by transducing lambda phages, e.g., λ*pgal*, φ*80*·*lac*, and λ*plac*, in plant cells (10–12). As a result, enzyme activity was indirectly demonstrated by longer survival of cultured plant cells growing on galactose or lactose medium, respectively, after infection with the corresponding transducing phage; the *z* gene product β-galactosidase was further identified by these authors with an immunochemical method (10, 11).

Specialized transducing phages have the great advantage that they can be used to isolate selected genes and replicate them in large quantities. Therefore, we also chose such a system for our studies of gene transfer into human cells. We used as gene donor the specialized transducing bacteriophage λ*plac* carrying the gene for β-galactosidase (14), and as recipient cells human skin fibroblasts from a patient with generalized gangliosidosis [GM_1-gangliosidosis Type I (15)] with a severe deficiency of β-galactosidase activity.

We infected the β-galactosidase-deficient fibroblasts with either the bacterial virus λ*plac* or with λ*plac* DNA. This plaque-forming *lac* transducing phage contains, besides the viral genes, a complete *z* gene, the gene for *Escherichia coli* β-galactosidase. Our aim was (*a*) to measure phage genome expression by detection of new β-galactosidase activity in the infected cells and (*b*) to determine whether the resulting enzyme activity is really *E. coli*-specific.

As control, uninfected GM_1 fibroblasts were used, as well as fibroblasts infected with the wild-type phage λ*W8*. We have detected a significant overall increase of the β-galactosidase activity in about 15% of the λ*plac*-infected cells. The same induced enzyme activity was significantly higher in cells after infection with λ*plac* DNA. Furthermore, it could be shown that the induced enzyme activity resembled that of *E. coli* β-galactosidase with respect to its immunochemical and chromatographic behavior.

MATERIALS AND METHODS

Cells. GM_1-gangliosidosis (GM_1) fibroblasts, with reduced β-galactosidase levels, from a patient with generalized gangliosidosis, were provided by W. Krone; normal human fibroblasts Wi 38, α_1, and HeLa cells, by H. Berthold and E. Petersen.

Culture Medium and Growth Conditions. Monolayer cultures were grown at 37° in 44 cm^2 rubber-stoppered tissue culture glass flasks in 20 ml of Eagle's minimum essential medium (MEM), containing 2.2 g/liter of sodium bicarbonate, 10% fetal calf serum (Gibco), 100 units/ml of penicillin, 50 μg/ml of streptomycin, and 0.292 g/liter of glutamine.

Media used were tested for bacterial and fungal contaminations by inoculation of Endo agar, charcoal and blood agar plates and Sabouraud agar plates with 0.2 ml of medium (*a*) before addition to the cells and (*b*) when cells were harvested. In addition, sterility controls of spent media from uninfected cells and infected cells (Table 1, Exps. 4 and 5) were performed by inoculation of 0.3 ml of medium into thioglycollate broth, serum broth, glucose broth, fresh tissue culture medium, and Sabouraud broth, and incubation for 10–14 days at 37° and at room temperature; Sabouraud broth was incubated at 28°. Subcultures of cell lines used were periodically monitored for mycoplasma contamination. Cells were plated in petri dishes, incubated for 24–48 hr at 37° in an atmosphere of 5% CO_2 in air. Then 2 μCi of [^{3}H]thymidine per ml was added, and after an incubation

Abbreviations: PBS, phosphate-buffered saline; MEM, Eagle's minimal essential medium; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

† We will use the term "infection" for cells that have been treated with the phage or its DNA.

time of 3 days autoradiographic detection of cytoplasmic incorporated tritiated thymidine was performed as described (16).

Preparation of Cell Extracts. Cells were trypsinized, washed twice with 0.9% NaCl, resuspended in 1 ml of 0.2 M sodium phosphate, pH 6.8 and sonicated three times for 10 sec at 20 W (Branson Sonifier B 12). After centrifugation at 12,000 × *g* for 60 min the supernatant was used for enzyme assays or stored at −70°.

Assay for β-Galactosidase. Measurements of β-galactosidase activity (β-D-galactoside galactohydrolase, EC 3.2.1.23; Boehringer) were carried out by a modification of the procedures described by Robins *et al.* and Norden *et al.* (17, 18). Thirty-five microliter aliquots of cell extract or *E. coli* β-galactosidase in assay buffer (0.5 mM 4-methylumbelliferone-β-D-galactopyranoside, Serva, 1 mM $MgCl_2$ in 0.2 M phosphate buffer, pH 6.8) were incubated for 30, 60, and 120 min at 37°. The reaction was stopped by addition of 1 ml of 0.5 M sodium carbonate buffer, pH 10.7 and the fluorescence was read in a spectral fluorimeter (Perkin Elmar, excitation wavelength 370 nm, emitting wavelength 450 nm). The protein concentration of the cell extracts was determined with the Lowry reaction (19).

Heat treatment of cell extracts in the presence of antiserum to *E. coli* β-galactosidase was performed by incubation of the extract for 20 min at 61°. Usually 5 μl of rabbit anti-*E. coli* β-galactosidase (diluted 1:50 in 0.2 M phosphate buffer, pH 6.8) were used for each assay. After cooling down to room temperature the substrate was added and β-galactosidase activity was determined as described before.

Isolation of Phage and Phage DNA. Transducing phage λ*plac* 5 (originating from J. Shapiro) containing a terminal part of the *lac* repressor gene *i*, a part of the *lac* permease gene *y*, the intact *lac* controlling region *p* and *o*, and the gene for β-galactosidase (*z* gene) (14) was heat induced from *E. coli* strain BMH 782, Δ (*lac*) *thi*$^-$ (λcI857S7 *plac* 5 *i*$^-$ *z*$^+$ *y*$^-$). Phage λ*W8* (λcI857S7) was isolated from strain DK 121 [W8K12*sup*$^+$ (λcI857S7)], a heat-inducible λ lysogen, obtained from D. Kamp. Strain BMH 783, (*lac*) *thi*$^-$ *supE*, was used as suppressor strain for the determination of the phage titers. All BMH strains were obtained from B. Müller-Hill.

Heat-induced phages were centrifuged on a stepwise CsCl gradient and banded several times (at least four times) on an equilibrium CsCl gradient. The titer of the purified phages, dialyzed against SM buffer (0.1 M NaCl, 10 mM $MgSO_4$, 10 mM Tris·HCl, pH 7.5, 0.01% gelatin), was determined by the plaque assay on BMH 783 and measured spectrophotometrically (1 absorbance unit at 260 nm corresponds to 4 × 10^{12} phage per ml). Before use the phage was dialyzed in phosphate-buffered saline (PBS) (20) containing 0.01 M $MgCl_2$, diluted into the same buffer, and passed through Millipore filters (pore size 0.45 μm).

Phage DNA was obtained by phenol extraction of the virus after heating for 10 min at 55° in the presence of 0.01 M EDTA. The DNA was precipitated three times with 70% ethanol in the presence of 0.05 M sodium acetate and dialyzed in Hepes buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.05 (21).

Treatment of GM_1-Gangliosidosis Fibroblasts with Phage. Exponentially growing gangliosidosis fibroblasts (0.5 to 1 × 10^6 cells) were infected in a manner similar to that described (2). Cells were rinsed twice with 5 ml of PBS containing 0.01 M $MgCl_2$. Millipore-filtered phages were added (λ*plac* or λ*W8* in 2 ml of PBS containing 0.01 M $MgCl_2$) at a multiplicity of 2 × 10^6 plaque-forming units per cell.

Table 1. Assays for β-galactosidase activity

Cell strain	β-Galactosidase activity* at pH 4.0	at pH 6.8	at pH 6.8†
Normal fibroblasts:			
Wi38	517.1		
α_1	95.4		
HeLa cells	32.4		
GM_1-gangliosidosis fibroblasts:			
(a) Uninfected	1.6	<0.4	<0.2
(b) Infected with phage λ*W8*		<0.4	<0.2
(c) Infected with phage λ*plac; Exp. no.*			
1		1.9	0.9
2		0.8	0.8
3		2.5	0.5
Infected with λ*plac* DNA			
4		182.0	178.0
5		348.0	223.0
6		5.6	3.1
7		1.4	0.9
(d) Infected with λ*W8* DNA		<0.4	<0.2

β-Galactosidase activities detected in normal cells (numbers reflect an average of two cell extracts from each cell line), β-galactosidase-deficient (GM_1-gangliosidosis fibroblasts (average of 13 cell extracts), and phage λ*plac*- and λ*plac*-DNA-infected GM_1-gangliosidosis fibroblasts.

* Enzyme activity is expressed in terms of nmol of 4-methylumbelliferone liberated in 60 min/mg of protein at 37°.

† β-Galactosidase activity after heat treatment in the presence of specific antibodies as described under *Materials and Methods.*

Virus-treated cells were incubated for 1 hr at 37° with occasional gentle shaking. After addition of 20 ml of tissue culture medium the cells were again incubated at 37° for 4 days and harvested for enzyme assay.

Treatment of GM_1-Gangliosidosis Fibroblasts with Phage DNA. β-Galactosidase-deficient fibroblasts (same cell density as for phage infection) were also used for inoculation with phage DNA (λ*plac* or λ*W8*). Cells pretreated with 0.4 ml of DEAE-dextran (150 μg/ml in 0.96% MEM without bicarbonate–0.2 M Tris, pH 7.35) at 37° for 30 min were incubated after removal of DEAE-dextran together with 20–30 μg of phage DNA in 2 ml of a Hepes–DNA–$CaCl_2$ mixture (125 mM) as described by Graham *et al.* (21). This DNA was allowed to absorb for 30–40 min with occasional gentle shaking at 37°. After the addition of 20 ml of tissue culture medium the cells were again incubated at 37° until harvested for enzyme assay. Following a modified procedure (30) in some experiments, the Hepes–DNA–$CaCl_2$ mixture was added directly to the cell medium. After 4 hr incubation at 37°, the medium was removed and replaced by 20 ml of fresh tissue culture medium.

DEAE-Cellulose Chromatography. Appropriate amounts of cell extract (0.01–0.04 mg of protein) and *E. coli* β-galactosidase were passed through DEAE-cellulose columns (1.0 × 0.6 cm or 1.0 × 0.34 cm) and eluted with a linear gradient of NaCl from 0 to 0.7 M in 0.01 M sodium phosphate buffer, pH 6.8. The columns were equilibrated with 0.01 M sodium phosphate, pH 6.8. Fractions containing 1 ml (column: 1.0 × 0.6 cm) or 0.5–0.075 ml (column: 1.0 × 0.34 cm) were col-

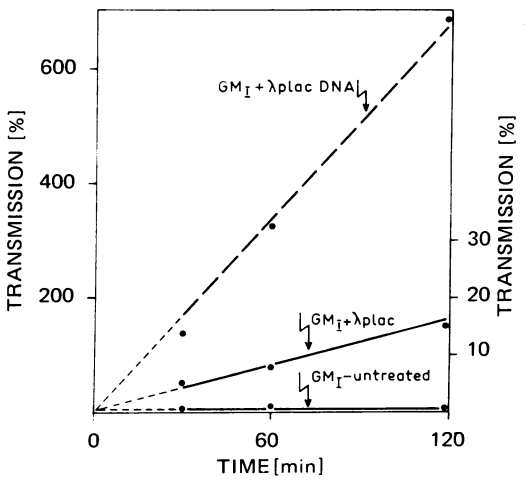

FIG. 1. Linearity of the β-galactosidase assay as a function of time performed at pH 6.8. Extracts were from uninfected GM$_I$ fibroblasts, λ*plac*-infected GM$_I$ fibroblasts (solid line, Exp. no 1, Table 1), and λ*plac*-DNA-infected GM$_I$ fibroblasts (broken line, Exp. no 4, Table 1). Enzyme activity is expressed in relative fluorescence intensities. 100% on the fluorimeter scale corresponds to 0.05 nM 4-methylumbelliferone. The right ordinate applies to the solid lines, the left one to the broken line. Enzyme assay was performed as described under *Materials and Methods*.

lected and immediately assayed for conductivity and β-galactosidase activity.

RESULTS

Induction‡ of β-galactosidase activity in GM$_I$ fibroblasts

β-Galactosidase Activity of GM$_I$-Gangliosidosis Fibroblasts at pH 4.0 and at pH 6.8. In contrast to the pH activity curve of *E. coli* β-galactosidase with its optimum at about pH 6.8, depending on the substrate and the salt conditions (22), the mammalian β-galactosidases demonstrate two optimums, a major one varying from pH 3.0 to 5.5 and a minor one at pH 6.6 (15, 18, 22, 23), again depending on experimental conditions. As shown in Table 1, GM$_I$-gangliosidosis fibroblasts show little activity at pH 4.0 and almost none at pH 6.8; *E. coli* β-galactosidase is inactive at pH 4.0 (Fig. 2). Increased activities after infection with λ*plac* could only be detected at pH 6.8 and not at pH 4.0. Since these induced activities corresponded to *E. coli* β-galactosidase in their immunochemical properties (see below) assays of β-galactosidase activity in subsequent infection experiments were performed at pH 6.8.

β-Galactosidase Activity in GM$_I$ Fibroblasts after Infection with λ*plac* and λ*W8*. Human β-galactosidase-deficient fibroblasts were infected with phage λ*plac* during exponential growth as described under *Materials and Methods*. Four days after infection they were assayed for β-galactosidase activity at pH 6.8. Higher enzyme activity—compared to activities of uninfected control cells—could be detected in three out of 19 experiments. Thus in about 15% of our experiments phage λ*plac* infection of GM$_I$ cells resulted in β-galactosidase induction in the deficient fibroblasts. β-Galactosidase activities were significantly higher than in the control experiments (Table 1, Fig. 1).

Under identical conditions β-galactosidase-deficient fibroblasts were also infected with the wild-type phage λ*W8*, lacking any genetic information for β-galactosidase. Enzyme tests of the cell extracts showed no difference in β-galactosidase activity compared to uninfected fibroblasts (Table 1). All assays for microbial contaminations (bacteria, fungi, and mycoplasma) carried out as described under *Materials and Methods* were negative.

‡ We will use the term "induced" for enzyme activities that arise after treatment of the cells with phage or phage DNA.

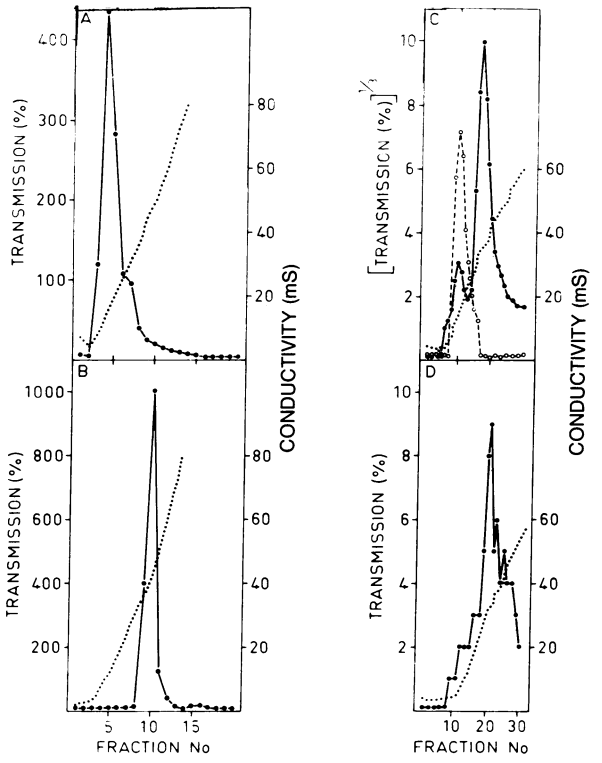

FIG. 2. Ion-exchange chromatography and assay for β-galactosidase activity and conductivity (. . . .) of collected fractions of normal human fibroblast extract (A), *E. coli* β-galactosidase (B) (column: 1.0 × 0.6 cm), a mixture of normal fibroblast extract and *E. coli* β-galactosidase (C), and λ*plac*-DNA-infected GM$_I$ fibroblast extract (D) (column: 1.0 × 0.34 cm) from Exp. no 4 (Table 1). In three experiments performed, conductivity for fibroblast β-galactosidase was found to be 14, 16, and 15 mS (average 15 mS) and for *E. coli* β-galactosidase 35, 42, and 36 mS (average 37 mS) (S = ohm^{-1}). Enzyme assay was performed at pH 6.8 and for (C) at pH 4.0 (O- - -O) and pH 6.8 (●—●) as described under *Materials and Methods*. Details of the chromatography on DEAE-cellulose are given in the *text*.

Treatment of GM$_I$ Fibroblasts with *E. coli* β-Galactosidase. In contrast to DNA preparations used for infection experiments (see below) phage λ*plac* preparations were contaminated with β-galactosidase even after several CsCl-gradient centrifugations of the virus. In Exps. 1 and 3 of Table 1 the contamination theoretically could account for about half or two thirds of the total β-galactosidase activity measured after infection, and in Exp. 2 for about 100% of the total activity. Therefore, GM$_I$ cells were treated with high amounts of *E. coli* β-galactosidase, enough to hydrolyze more than 300.0 nmol of 4-methylumbelliferone-β-D-galactopyranoside in 60 min/mg of cellular protein at 37°. Incubation of the cells for 4 days under the same conditions used in the infection experiments did not lead to *E. coli*-specific β-galactosidase activity in cell extracts when tested (data not shown).

β-Galactosidase Activity in GM$_I$ Fibroblasts after Infection with λ*plac* and λ*W8* DNA. β-Galactosidase activity could not be detected in λ*plac* DNA preparations, in con-

trast to phage λ*plac* preparations. The λ*plac*-DNA-infected deficient human fibroblasts were harvested 72–96 hr after infection. In experiments with DEAE-dextran pretreatment the cells looked stressed, showed cytoplasmatic granularity, and some cells rounded up and became detached from the flask wall (Table 1, Exps. 4, 5, and 6) after 72 hr. Under these conditions cells were harvested 72 hr after infection. In four out of 16 infection experiments with λ*plac* DNA a high increase of the β-galactosidase activity could be detected in some cell extracts. Enzyme activities of positive experiments were measured as reaching from 1.4 to 348.0 nmol of hydrolyzed substrate in 60 min/mg of protein at 37° (Table 1). Compared to phage-infected cells, induced β-galactosidase activity was much higher in phage-DNA-infected cells, and in addition, the DNA-induced activity levels differed considerably. In control experiments β-galactosidase-deficient fibroblasts were treated with λ*W8* DNA under identical conditions. No difference in β-galactosidase activity compared to uninfected fibroblasts could be detected.

Characterization of β-galactosidase activity induced by phage λ*plac* and λ*plac* DNA

Protection of Enzyme Activity by *E. coli*-Specific Antibodies. Recent experiments demonstrated that *E. coli* β-galactosidase can be protected against heat inactivation by *E. coli* β-galactosidase antibodies (24). Therefore, it could be assumed that *E. coli* β-galactosidase, if present in fibroblasts, should remain active in the presence of anti-*E. coli* β-galactosidase after heat inactivation of the mammalian β-galactosidases. Experiments with cell extracts of β-galactosidase-deficient cells after addition of *E. coli* β-galactosidase and specific antibodies confirmed this assumption. Protection of *E. coli* β-galactosidase activity varied from about 50% to 100% (data not shown). This assay was used for our experiments to discriminate induced *E. coli* β-galactosidase activity in GM_I cells from the mammalian specific β-galactosidase activity. Therefore, enzyme assays of all cell extracts, infected or uninfected, were also carried out routinely after heat treatment of the cell extracts in the presence of anti-*E. coli* β-galactosidase antibodies (for details see *Materials and Methods*).

Heat inactivation for 20 min at 61° of fibroblast extracts, from cells either uninfected or infected with either phage λ*plac* or λ*plac* DNA, resulted in a complete loss of β-galactosidase activity. When heat inactivation was carried out in the presence of rabbit anti-*E. coli* β-galactosidase, protection of enzyme activity at pH 6.8 was higher than 20% in either of those experiments where phage λ*plac*- or λ*plac*-DNA-infected fibroblast extracts showed induced activity before heat inactivation (Table 1). This suggests and makes it rather likely that the heat-resistant β-galactosidase activity was *E. coli*-specific.

Separation of Fibroblast and Induced *E. coli*-Specific β-Galactosidase Activity by Ion-Exchange Chromatography. As demonstrated in Fig. 2 the two β-galactosidase activities from human fibroblasts and *E. coli* could be well separated on DEAE-cellulose columns. Cellular and *E. coli* enzyme resolved from each other and eluted at different ionic strengths as compared by elution position or conductivity measurements at point of emergence after chromatography. It could further be demonstrated that heat treatment of the eluate in the presence of anti-*E. coli* β-galactosidase resulted in the disappearance of the fibroblast β-galactosidase activity peak, while the *E. coli* β-galactosidase peak remained in the same position (data not shown). Thus we had an additional confirmation of the validity of the *E. coli* β-galactosidase activity protection test used here.

When extracts of the GM_I fibroblasts with λ*plac*-induced activity were run on DEAE-cellulose columns under the same conditions, induced enzyme activity was detected at the position where *E. coli* β-galactosidase is eluted (Fig. 2). Heat treatment of the eluate in the presence of anti-*E. coli* β-galactosidase did not destroy this enzyme activity. From these data it was concluded that λ*plac*-induced enzyme activity in the GM_I fibroblasts is not cell- but *E. coli*-specific.

DISCUSSION

It could be demonstrated that incubation of human β-galactosidase-deficient fibroblasts with transducing phage λ*plac* or with λ*plac* DNA led to higher β-galactosidase levels of cell extracts. Phage infection gave increased β-galactosidase activities in about 15% of the performed experiments and phage DNA in about 25%. Furthermore phage λ*plac*-DNA-induced β-galactosidase activities in deficient cells were much higher than those obtained after phage λ*plac* infection. Phage λ*plac* codes for an *E. coli*-specific β-galactosidase and, therefore, the induced enzyme activity should not be cell-specific but *E. coli*-specific. Heat inactivation tests of the induced enzyme activities in the presence of *E. coli*-specific antibodies and ion-exchange chromatography presented the evidence that the induced β-galactosidase activity is under these conditions indistinguishable from that of β-galactosidase from *E. coli*.

We have shown that induced enzyme activities were not due to β-galactosidase contamination of phage or phage DNA preparations or other materials used, and in all our control experiments bacterial, fungal, and mycoplasma contaminations could not be detected. Our results lead us to the conclusion that, of the offered prokaryotic genetic information, at least that of gene *z* had been transferred to and expressed in eukaryotic cells. The occurrence of this type of gene transfer and the acceptance by the mammalian cellular machinery for transcription and translation is in agreement with similar findings by Merril *et al.* (2). From the control experiments it is rather unlikely that the contamination of phage λ*plac* with β-galactosidase contributed significantly to the detected activities in phage-infected cells.

There is no good explanation why in our gene transfer experiments enzyme activity occurred only in a certain percentage and why individual experiments show a broad variation of induced enzyme levels. However, cellular susceptibility to offered genetic material may vary from one experiment to another, leaving the identity of the variables as yet undetermined.

The phenomenon that phage DNA is much more efficient in inducing enzyme activities compared to intact phage particles agrees with published results (2). One assumption might be that DNA is taken up better by cells than whole phage particles and/or that DNA is more easily available for transcription and translation by the cellular apparatus. The finding of phage-specific RNA in phage-infected cells (2, 3) will have to be proven for our system. The possible persistence over several cell generations of phage genetic information in eukaryotic cells (2, 11, 12) can also be the object of further investigations. A test for lactose utilization of infected β-galactosidase-deficient cells was not performed. Preliminary experiments suggested that the deficient fibroblasts used here still contain enough β-galactosidase so that lactose will be accepted as the sole source of hexose (Horst and Kluge, unpublished results).

Phage λ is probably the most intensively studied temperate *E. coli* phage (25), and one of the best-known regulatory mechanisms of microorganisms concerns the *lac* operon of *E. coli* (25–29). Thus the introduction of the *lac* operon into mammalian cells and its expression there may contribute to our understanding of regulatory mechanisms in eukaryotic cells.

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1. Sander, E. M. (1967) *Virology* **33,** 121–130.
2. Merril, C. R., Geier, M. R. & Petricciani, J. C. (1971) *Nature* **233,** 398–400.
3. Geier, M. & Merril, C. R. (1972) *Virology* **47,** 638–643.
4. Anker, P. & Stroun, M. (1972) *Science* **178,** 621–623.
5. Morrison, T. G. & Lodish, H. F. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 315–319.
6. Richter, D., Herrlich, P. & Schweiger, M. (1972) *Nature New Biol.* **238,** 74–76.
7. Aviv, H., Boime, I., Loyd, B. & Leder, P. (1972) *Science* **178,** 1293–1295.
8. Schreier, M. H., Staehelin, T., Gesteland, R. F. & Spahr, P. F. (1973) *J. Mol. Biol.* **75,** 575–578.
9. Carlson, P. S. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 598–602.
10. Doy, C. H., Gresshoff, P. M. & Rolfe, B. G. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 723–726.
11. Doy, C. H., Gresshoff, P. M. & Rolfe, B. G. (1973) *Nature New Biol.* **244,** 90–91.
12. Johnson, C. B., Grierson, D. & Smith, H. (1973) *Nature New Biol.* **244,** 105–107.
13. Ledoux, L. & Huart, R. (1974) *Nature* **249,** 17–21.
14. Ippen, K., Shapiro, J. A. & Beckwith, J. R. (1971) *J. Bacteriol.* **108,** 5–9.
15. O'Brien, J. S. (1972) in *The Metabolic Basis of Inherited Disease,* eds. Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S. (McGraw-Hill Book Co., New York), pp. 639–662.
16. Studzinski, G. P., Giethy, J. F. & Cholon, J. J. (1973) *In Vitro* **8,** 466–472.
17. Robins, E., Hirsch, H. E. & Emmons, S. S. (1968) *J. Biol. Chem.* **243,** 4246–4252.
18. Norden, A. G. W. & O'Brien, J. S. (1973) *Arch. Biochem. Biophys.* **159,** 383–392.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
20. Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99,** 183–199.
21. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52,** 456–467.
22. Wallenfels, K. & Weil, R. (1974) in *The Enzymes,* ed. Boyer, P. D. (Academic Press, New York and London), pp. 617–663.
23. Hultberg, B. & Öckerman, P. A. (1972) *Clin. Chim. Acta* **39,** 49–58.
24. Melchers, F. & Messer, W. (1970) *Biochem. Biophys. Res. Commun.* **40,** 570–575.
25. Hershey, A. D., ed. (1971) *The Bacteriophage Lambda* (Cold Spring Harbor Laboratory, New York, N.Y.).
26. Jacob, F. & Monod, J. (1961) *J. Mol. Biol.* **3,** 318–356.
27. Gilbert, W. & Müller-Hill, B. (1966) *Proc. Nat. Acad. Sci. USA* **56,** 1891–1898.
28. Beyreuther, K., Adler, K., Geisler, N. & Klemm, A. (1970) *Proc. Nat. Acad. Sci. USA* **70,** 3576–3580.
29. Gilbert, W. & Maxam, A. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 3581–3584.
30. Groneberg, J., Brown, D. T. & Doerfler, W. (1975) *Virol.* **64,** 115–131.