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Complementation of the UV-sensitive phenotype of a xeroderma pigmentosum human cell line by transfection with a cDNA clone library

(gene transfer/DNA repair)

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ABSTRACT **In previous work, a xeroderma pigmentosum cell line belonging to complementation group C was established by transformation with origin-defective simian virus 40. We now report the complementation of the UV sensitivity of this cell line by gene transfer. A human cDNA clone library constructed in a mammalian expression vector, and itself incorporated in a λ phage vector, was introduced into the cells as a calcium phosphate precipitate. Following selection to G418 resistance, provided by the *neo* gene of the vector, transformants were selected for UV resistance. Twenty-one cell clones were obtained with UV-resistance levels typical of normal human fibroblasts. All transformants contained vector DNA sequences in their nuclei. Upon further propagation in the absence of selection for G418 resistance, about half of the primary transformants remained UV-resistant. Secondary transformants were generated by transfection with a partial digest of total chromosomal DNA from one of these stable transformants. This resulted in 15 G418-resistant clones, 2 of which exhibited a UV-resistant phenotype. The other primary clones lost UV resistance rapidly when subcultured in the absence of G418. Importantly, several retained UV resistance under G418 selection pressure. The acquisition of UV resistance by secondary transformants derived by transfection of DNA from a stable primary transformant, and the linkage between G418 and UV resistances in the unstable primary transformants, strongly suggests that the transformants acquired UV resistance through DNA-mediated gene transfer and not by reversion.**

Xeroderma pigmentosum (XP) is an autosomal recessive human disease manifested as an extreme sensitivity to UV light resulting in a very high incidence of skin cancer and, in many patients, neurological abnormalities. Cells from XP patients are defective in the excision repair of pyrimidine dimers in DNA and are extremely sensitive to killing by UV radiation. Nine complementation groups (A–I) defective in excision repair have been defined, whereas the XP "variant" appears to have normal levels of excision repair but is impaired in postreplication repair (1). The molecular basis for the different XP mutations has not been characterized—neither genes nor gene products have been identified. Stable corrections of the UV sensitivity of simian virus 40 (SV40)-transformed XP cell lines of complementation group A (XP-A cell lines) by transfection with normal human DNA (2) or following fusion with either x-ray-irradiated Chinese hamster ovary (CHO) cells (3) or microcells containing a single human chromosome (4) were reported. Transient complementation of excision-repair defects of XP primary cell lines was achieved either by microinjecting mRNA from normal human cells into XP-A and XP-G cells (5) or by introducing protein extracts from normal human cells into XP-A cells (6, 7) or into cells of all other XP complementation groups (8). Another approach for isolation of human excision-repair genes has been the stable complementation of excision-deficient CHO cells produced in the laboratory. This led to molecular cloning (9, 10) of a human gene (*ERCC1*) that complements group-2 CHO mutants, and its mapping to human chromosome 19 (10–12). The relationship of this gene, if any, to human DNA-repair genes defective in XP is under investigation.

One of the major obstacles in experiments aiming at stable complementation of XP cells is the scarcity of immortalized XP cell lines. Therefore, using for transfection an SV40 DNA fragment that contains a defective origin of replication but a functional gene encoding large tumor (T) antigen, we immortalized two XP-C cell lines (13). These cell lines retain the high sensitivity to UV irradiation and, unlike many of the SV40-transformed human fibroblasts, show very few chromosomal changes, although the transfecting SV40 DNA is integrated into cellular DNA sequences. We now report the complementation by gene transfer of the UV sensitivity of one of these established XP-C cell lines.

METHODS

Cell Culture, Transfections, and Selection of Transformants. The XP-C recipient cell line GM2096-SV3 had been described (13). This cell line and all cells derived from it by transfection, as well as GM637 (SV40-transformed human fibroblasts), were maintained in Dulbecco's modified Eagle's medium containing penicillin, streptomycin, gentamicin, and 10% fetal bovine serum (Biological Industries, Beth Haemek, Israel). Cells were routinely subcultured every 3–4 days.

Transfection with the recombinant phage λNMT-pcD-GM637 was as described (14) with the single exception that the "expression period" following washing of the phage particles was extended to 2 days. Then the cells were trypsinized and replated at 1:5 dilution. Selection for G418 resistance was instituted 12–16 hr later by the addition of antibiotic G418 (GIBCO) at 400 μg/ml (15, 16). About 2½ weeks later, G418-resistant ($G418^R$) colonies were trypsinized and replated. The next day the cells were subjected to a UV-irradiation protocol, employing 4J/m², four times at 1- to 2-day intervals. All operations were conducted in gold light (500–700 nm). Two weeks following the last UV irradiation, the surviving colonies were picked and grown to mass culture for further characterization. During the UV selection and

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Abbreviations: XP, xeroderma pigmentosum; XP-*x* cell line, XP cell line of complementation group *x*; SV40, simian virus 40; $G418^R$, G418-resistant; UV^R, UV-resistant.

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later, during propagation of the surviving cell clones, no selection for G418 resistance was employed.

For generation of secondary transformants, total chromosomal DNA extracted from primary transformants was partially digested with *Mbo* I restriction endonuclease to fragments with an average length of 50 kilobases (kb). These were transfected into the GM2096-SV3 cells (total number 5×10^6) as a calcium phosphate precipitate (17), at 20 μg for 10^6 cells, followed by a 1-min glycerol shock after 4 hr (18). About 48 hr later, the cells were trypsinized and replated at 1:20 dilution. Following selection with G418, several among the resistant colonies were individually picked, grown to mass culture, and assayed for UV resistance.

Assay of UV Resistance. Cells (10^5) were plated in 60-mm plastic tissue culture dishes, and the next day they were irradiated from above with a germicidal lamp (the UV dose rate was 0.17 J/m^2 per sec, as measured by a UVX digital radiometer, Ultra Violet Products, San Gabriel, CA). The cells were cultured in the growth medium at 37°C and 5 days later the number of surviving cells was determined after trypsinization and trypan blue staining. The relative survival value for each dose is the mean of duplicate determinations. It was determined by dividing the number of live cells on UV-irradiated dishes by the corresponding number of cells on unirradiated dishes.

RESULTS

Complementation of the UV Sensitivity of an XP-C Cell Line. In an attempt to complement the UV sensitivity of our XP-C established cell line GM2096-SV3 (13), we introduced into it a human cDNA library from SV40-transformed human fibroblasts (GM637), constructed in the pcD mammalian expression vector (19). The pcD-GM637 human cDNA library is in turn incorporated in the bacteriophage λ vector NMT (14). λNMT contains a transcription unit of *neo*, a dominant selectable marker that renders mammalian cells resistant to the toxic drug G418. The *neo* gene is located in λNMT 0.25 kb from the pcD-GM637 library insertion site. Transfection was accomplished with λ phage particles under conditions similar to those specified before (14). About 2×10^7 cells were transfected, followed by selection for G418 resistance. The efficiency of transformation to G418 resistance was 2.5×10^{-4}. The surviving colonies were trypsinized and replated and then were subjected to several consecutive UV irradiations at intervals of 1–2 days. The UV dose employed (4 J/m^2) is lethal to the GM2096-SV3 recipient cells but causes little damage to normal human fibroblasts. The surviving colonies were isolated individually and grown to mass culture. The UV sensitivity of the isolated transformants was assayed by UV-survival measurements as indicated in *Methods*. Twenty-one UV-resistant (UV^R) cell clones were isolated, a partial list of which is shown in Table 1. Inspection of their initial levels of UV survival shows that most clones gained UV-resistance levels similar to those of normal human fibroblasts transformed by SV40 (GM637), exceeding that of the parental cells (GM2096-SV3) by 3 orders of magnitude. Out of 20 dishes initially transfected, UV^R transfectants were derived from dish no. 5 and a pool of G418^R transformants grown in dishes 17–20. These data suggest that there are at least two independent UV^R cell transformants. Control experiments employing the GM2096-SV3 cell line by itself, or after its transfection with the λNMT phage vector alone, followed by G418 selection (in the latter) and UV selections, did not give rise to UV^R colonies.

Stability of UV^R Phenotype in Primary Transformants. Following the initial identification of UV^R transformants, these cell clones were tested periodically for retention of the UV^R phenotype while subcultured in the absence of selection for G418 resistance. As shown in Table 1, most of the transformants initially derived from dishes 17–20 (except for clones 17-20/8 and 17-20/9) retained the UV^R phenotype during subsequent passaging, whereas all five UV^R transformants isolated from dish 5 showed decreased UV resistance. Particularly interesting are the cases of transformants 5/1, 5/5, and 5/10, in which instability of UV resistance was accompanied by loss of G418 resistance. We therefore reinstituted G418 selection in these cell lines at the earliest passage available. Selection for *neo* expression, resulting in

Table 1. UV resistance of XP-C primary transformants

Clone*	Passage no. since clone's establishment	Fraction of cells surviving UV dose	
		2 J/m^2	3 J/m^2
GM637 (normal)	—	1.0	0.44
GM2096-SV3 (XP-C)	—	0.0007	<0.0003
Transformants			
17-20/1	5	0.94	0.88
	24	0.83	0.37
	55	0.60	0.40
17-20/2	2	0.72	0.70
	24	0.66	ND
	55	0.65	0.40
17-20/3	3	0.93	0.93
	11	ND	0.87
	50	0.83	0.55
17-20/4	2	0.83	0.66
	26	0.62	0.62
	74	ND	0.50
17-20/5	2	0.88	0.64
	24	0.70	0.60
	75	ND	0.50
17-20/6	2	0.80	ND
	16	0.84	0.80
	32	0.93	0.80
17-20/8	6	0.30	0.24
	16	0.14	0.07
	25	<0.0003	<0.0003
17-20/9	3	0.85	0.55
	12	0.52	0.33
	25	<0.10	<0.10
17-20/10	12	0.80	0.50
5/1	4	0.45	0.34
	6	0.20	0.15
	21	0.04	0.01
5/1-G418^R	12	0.50	0.38
	28	0.26	0.20
	50	0.20	0.15
5/4	2	0.15	ND
	8	0.15	0.05
5/5	3	0.57	0.57
	10	0.05	0.04
5/5-G418^R	8	0.37	ND
	13	0.25	0.27
	69	0.30	0.20
5/9	2	0.42	0.28
	10	0.08	0.02
5/10	3	0.75	0.62
	10	0.33	0.20
	18	0.08	0.07
5/10-G418^R	6	0.37	0.33
	14	0.52	0.34
	19	0.58	0.47
	69	0.50	0.35

ND, not done.

*GM637 is a normal human fibroblast line transformed with SV40. GM2096-SV3 is an SV40-transformed line of XP-C fibroblasts; UV^R transformant clones were obtained by transfection of this XP-C line with a GM637 cDNA (λNMT-pcD-GM637) library.

the three cell clones 5/1-G418^R, 5/5-G418^R, and 5/10-G418^R, also stabilized the UV^R phenotype (Table 1). Such a rapid loss of G418 and UV resistances cannot be attributed to reversion, but rather to the frequent instability of transduced foreign DNA, as indicated by Axel and coworkers (20). Moreover, the stabilization of UV resistance by G418 selection reinforced a linkage between the resistances to UV and G418 in these three cell transformants, implying that UV resistance was acquired by gene transfer and not by reversion.

Transfected DNA in High and Low Molecular Weight Nuclear DNA of Transformed Cells. As a prelude to molecular cloning of the cDNA sequences that conferred UV resistance to the XP-C cell line, we looked for the localization of the transfected DNA in the cell. Fig. 1 shows Southern blots (21) of *Eco*RI-cleaved high molecular weight DNA from different transformants after hybridization with ^{32}P-labeled *neo* DNA. Most transformants contained at least several copies of the *neo* gene integrated in the high molecular weight, ostensibly chromosomal, DNA of the cell clones. However, clone 5/10-G418^R contained *neo* in only one *Eco*RI DNA fragment, 30 kb in length. Clone 17-20/4 did not have any high molecular weight DNA fragment containing *neo*; neither, as expected, did the control cell line HeLa and the recipient cell line GM2096-SV3.

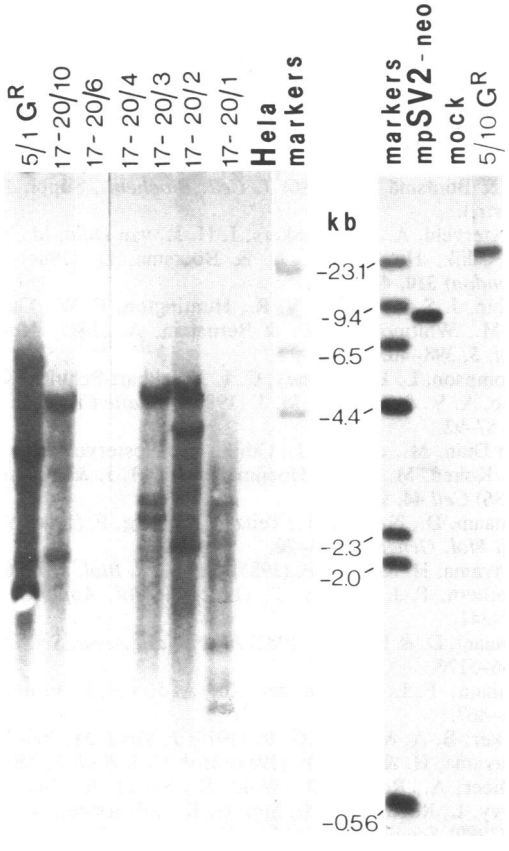

FIG. 1. *neo* sequences derived from λNMT-pcD-GM637 in the high molecular weight DNA extracted from UV^R XP-C transformants. Cell DNAs (20 μg) derived from GM2096-SV3 (mock), its UV^R transformants, and HeLa cells were prepared (22), digested to completion with the restriction endonuclease *Eco*RI, electrophoresed in 0.8% agarose, and transferred [after mild depurination (23)] to nitrocellulose paper (21). The blots were hybridized with ^{32}P-labeled (24) 1.0-kb *neo* DNA segment bounded by restriction sites *Sma* I and *Bgl* II (25). Included as positive control is mpSV2-neo plasmid DNA (16). Size markers were fragments produced by cleavage of λ phage DNA with *Hin*dIII and end-labeled with ^{32}P. G^R indicates the G418^R cell lines.

Since the UV^R transformants as human cells are semipermissive for SV40 DNA replication, and also synthesize SV40 large tumor (T) antigen due to their prior transformation/immortalization, they can support the autonomous replication of transfected DNA elements containing the SV40 origin of DNA replication. We therefore looked in the UV^R transformants for the occurrence of *neo* or SV40 early promoter sequences in autonomously replicating DNA of low molecular weight. Hybridization to Southern blots of low molecular weight DNA present in Hirt extracts (26) of transformed clones 5/1-G418^R, 5/5-G418^R, 17-20/2, 17-20/5, and 17-20/6 showed (Fig. 2) *neo*-containing autonomously replicating plasmids. In contrast, Hirt extracts derived from clones 5/10-G418^R, 17-20/1, and 17-20/3 or from GM2096-SV3 recipient cells did not contain sequences that hybridized with the *neo* probe. Hybridization of the same DNA blots with a ^{32}P-labeled SV40 early promoter probe (the fragment between the *Bgl* I and *Kpn* I restriction sites) revealed that clone 5/10-G418^R did not contain autonomously replicating DNA having homology to the SV40 early promoter, whereas clone 17-20/1, which (like 5/10-G418^R) lacked *neo* plasmids, had three plasmids with the SV40 sequence (data not shown).

UV^R Secondary Transformants. It could be argued that those transformants with a stable UV^R phenotype arose by spontaneous or UV-induced reversion. Although control experiments yielded no UV^R cell clones, we set out to test this argument by trying to obtain secondary UV^R transformants without the use of UV irradiation for selection. Total genomic DNA of transformant 17-20/6, which had a low number of *neo* copies in its nuclei (Figs. 1 and 2), was partially digested with *Mbo* I restriction endonuclease to fragments averaging 50 kb in length; and these fragments were transfected into GM2096-SV3 cells. Fifteen colonies selected for

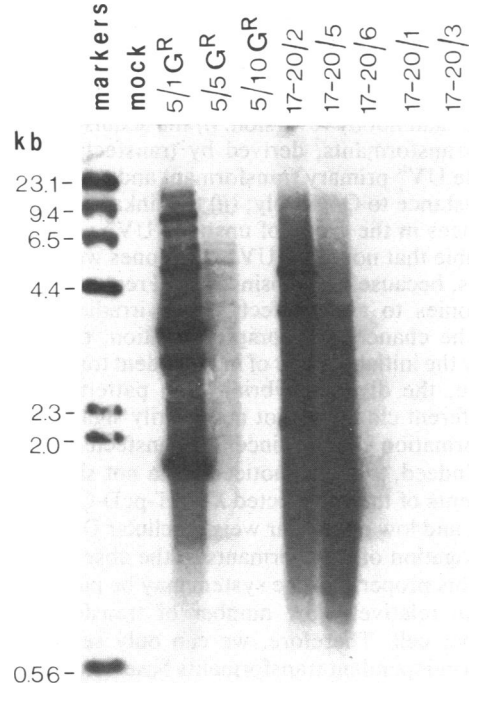

FIG. 2. *neo* sequences derived from λNMT-pcD-GM637 DNA in low molecular weight, autonomously replicating DNA extracted from UV^R XP-C transformants. Each lane contained Hirt supernatant (26) from 1.5 × 10^7 cells, except the 5/5 G^R lane (0.75 × 10^7 cells). Recipient cell line GM2096-SV3 (mock) served as negative control. Electrophoresis was done in 1.0% agarose; transfer and hybridization to *neo* DNA probe were as in Fig. 1. Size markers were *Hin*dIII fragments of λ phage DNA, labeled with ^{32}P at the ends. G^R indicates the G418^R cell lines.

Table 2. UV resistance of XP-C secondary transformants

Clone	Fraction of cells surviving UV dose	
	1.5 J/m²	2.0 J/m²
GM637	1.0	1.0
GM2096-SV3	0.004	0.0008
17-20/6-2/24	0.15	0.04
17-20/6-3/9	0.12	0.03

Secondary transformants were produced by transfecting GM2096-SV3 with a partial *Mbo* I digest of DNA from primary transformant 17-20/6.

G418 resistance (due to expression of endogenous *neo* genes) were isolated and grown for further characterization. Of these, 13 were shown by UV-survival measurements to be as UV-sensitive as the recipient cell line GM2096-SV3. However, two independent transformants (derived of different transfected plates) acquired partial but significant UV-resistance levels, 30- to 50-fold higher than the parental cell line GM2096-SV3 (Table 2). Since no UV selection was employed in the generation of the secondary transformants, and the proportion of UV^R clones among the $G418^R$ ones (2 out of 15) was relatively high, this result indicates that these UV^R clones arose due to DNA-mediated gene transfer and not by spontaneous or UV-induced reversions. Even though transfection was carried out with fragments of ≈50 kb, further experiments are needed to determine whether *neo* is physically linked to the DNA element that provides UV resistance.

DISCUSSION

Transduction of an immortalized XP-C cell line with a cDNA library incorporated in a mammalian expression vector led to complementation of the UV sensitivity of the recipient cells. Two lines of evidence strongly suggest that the XP-C transformants acquired UV resistance by DNA-mediated gene transfer and not by reversion: (*i*) the acquisition of UV^R secondary transformants, derived by transfection of DNA from a stable UV^R primary transformant and selected on the basis of resistance to G418 only; (*ii*) the linkage of G418 and UV resistances in the group of unstable UV^R clones.

It is possible that not all 21 UV^R cell clones were independent isolates, because we trypsinized and replated the transformed colonies to allow effective UV irradiation. While increasing the chances for complementation, this protocol may amplify the initial number of independent transformants. Furthermore, the distinct hybridization patterns observed with the different clones do not necessarily signify independent transformation events, since the transfected DNA may rearrange. Indeed, we have noticed (data not shown) DNA rearrangements of the transfected λNMT-pcD-GM637 DNA in both high and low molecular weight cellular DNA, at least during propagation of transformants in the absence of G418 selection. This property of the system may be partly responsible for the relatively low number of transfected DNA molecules per cell. Therefore, we can only set a minimal value of two independent transformants based on the number of initially transfected cell dishes from which UV^R transformants originated, indicating a minimal efficiency of transformation to UV resistance of 10^{-7}. Unlike transfection with genomic DNA fragments, our transfection experiments introduced DNA in which the elements that drive transcription and posttranscriptional processing are of nonhuman origin, being derived from SV40. Yet, apparently, they are recognized sufficiently well so that the expression of one or a few integrated cDNA copies per cell provides a wild-type level of UV resistance.

The complementing cDNA library was constructed with mRNAs from the SV40-transformed human fibroblast line GM637, itself not UV-irradiated or chemically treated. This indicates that, at least in SV40-transformed human fibroblasts, there is a constitutive level of mRNA that can complement the particular DNA-repair defect. Based on the nomenclature proposed at the 7th Human Gene Mapping Conference (27) we term the repair cDNA described here XPCC (xeroderma pigmentosum group C-complementing). In transformant $5/10\text{-}G418^R$, the resistances to G418 and UV are linked. As this transformant has also a single integration site for *neo* sequences, confined to the high molecular weight DNA of the cell, it seems a suitable candidate for rescue and cloning of the repair cDNA.

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1. Friedberg, E. C. (1985) *DNA Repair* (Freeman, New York), pp. 506–525.
2. Takano, T., Noda, M. & Tamura, T. (1982) *Nature (London)* **296**, 269–270.
3. Karentz, D. & Cleaver, J. E. (1986) *Mol. Cell. Biol.* **6**, 3428–3432.
4. Schultz, R. A., Saxon, P. J., Glover, T. W. & Friedberg, E. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4176–4179.
5. Legerski, R. J., Brown, B. B., Peterson, C. A. & Robberson, D. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5676–5679.
6. De Jonge, A., Vermeulen, W., Klein, B. & Hoeijmakers, J. (1983) *EMBO J.* **2**, 637–641.
7. Yamaizumi, M., Sugano, T., Asahina, H., Okada, Y. & Uchida, T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1476–1479.
8. Hoeijmakers, J. H. J., van Duin, M., Westerveld, A., Pastink, A. & Bootsma, D. (1986) *J. Cell. Biochem.*, Suppl. 10C, 29 (abstr.).
9. Westerveld, A., Hoeijmakers, J. H. J., van Duin, M., de Wit, J., Odijk, H., Pastink, A. & Bootsma, D. (1984) *Nature (London)* **310**, 425–429.
10. Rubin, J. S., Prideaux, V. R., Huntington, F. W., Dulhanty, A. M., Whitmore, G. F. & Bernstein, A. (1985) *Mol. Cell. Biol.* **5**, 398–405.
11. Thompson, L. H., Mooney, C. L., Burkhart-Schultz, K., Carrano, A. V. & Siliciano, M. J. (1985) *Somatic Cell Mol. Genet.* **11**, 87–92.
12. van Duin, M., de Wit, J., Odijk, H., Westerveld, A., Yasui, A., Koken, M. A. M., Hoeijmakers, J. H. J. & Bootsma, D. (1986) *Cell* **44**, 913–923.
13. Canaani, D., Naiman, T., Teitz, T. & Berg, P. (1986) *Somatic Cell Mol. Genet.* **12**, 13–20.
14. Okayama, H. & Berg, P. (1985) *Mol. Cell. Biol.* **5**, 1136–1142.
15. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
16. Canaani, D. & Berg, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5166–5170.
17. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
18. Parker, B. A. & Stark, G. R. (1979) *J. Virol.* **31**, 360–369.
19. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 280–289.
20. Pellicer, A., Robins, D., Wold, B., Sweet, R., Jackson, J., Lowy, I., Roberts, J. M., Sim, G. K., Silverstein, S. & Axel, R. (1980) *Science* **209**, 1414–1422.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
22. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
23. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
24. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
25. Beck, E., Ludwig, G., Averswald, E. A., Reirs, B. & Schaller, H. (1982) *Gene* **19**, 327–336.
26. Hirt, B. (1967) *J. Mol. Biol.* **26**, 365–369.
27. Shows, T. B., McAlpine, P. J. & Miller, R. J. (1984) *Cytogenet. Cell Genet.* **37**, 340–390.