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Recombination and Ligation of Transfected DNA in CHO Mutant EM9, Which Has High Levels of Sister Chromatid Exchange

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Transformation frequencies were measured in CHO mutant EM9 after transfection with intact or modified plasmid pSV2-*gpt*. The mutant and wild-type strain behaved similarly under all conditions except when homologous recombination was required to produce an intact plasmid. Therefore, the defect of the mutant which renders it slow in DNA strand break rejoining and high in sister chromatid exchange induction reduces its ability to recombine foreign DNA molecules.

Recombination of genomic sequences in mammalian cells is important in development and differentiation (25, 26), the generation of sister chromatid exchange (14), and chromosomal translocations in cancer (41), among other important cellular processes. However, the mechanisms involved in recombination in mammalian cells have not been elucidated to the extent that they have been in procaryotes and simple eucaryotes (22, 30). Recently, numerous studies have described systems for the study of recombination and ligation in mammalian cells. These systems generally involve the addition of foreign DNAs, such as animal viruses (38–40) or recombinant plasmids with selectable markers (5, 6, 17, 21, 27), and have shown that mammalian cells are able to efficiently reconstitute intact genes from gene fragments via homologous or nonhomologous recombination.

In the present study, transformation to mycophenolic acid resistance of CHO mutant EM9 was measured after transfection with intact or modified pSV2-*gpt*, which codes for bacterial guanosine phosphoribosyltransferase (18, 19), to assess integration, ligation, and recombination frequencies of the mutant compared with the wild-type strain, AA8 (36). Mutant EM9 has been characterized as having enhanced sensitivity to some, but not all, alkylating agents (9, 33, 37), slight sensitivity to X-irradiation (37), high base-line and induced sister chromatid exchange frequencies, and hampered ability to rejoin DNA strand breaks (33), making it an interesting candidate for the study of processing of exogenous DNA.

A diagram of pSV2-*gpt* and relevant restriction sites is shown in Fig. 1A. For transfection experiments, DNA was calcium phosphate precipitated (35) and added to monolayers (2×10^6 cells per dish) of AA8 and EM9 cells obtained from suspension cultures grown in alpha-MEM (KC Biologicals) supplemented with 10% fetal bovine serum (Flow Laboratories, Inc.) (alpha-MEMFBS) as described previously (34). After DNA exposure, the cell monolayers were rinsed twice with phosphate-buffered saline and incubated in alpha-MEMFBS for 24 h. They were then transferred to roller tubes for suspension growth. After the appropriate time for expression of the Gpt phenotype, cells were counted and plated for plating efficiency measurements in alpha-MEMFBS (300 cells per dish) or for selection (10^5 to 10^6 cells per dish) in MAXTA medium (alpha-MEMFBS supplemented with 10 μg of mycophenolic acid per ml, 0.25 μg of aminopterin per ml, 250 μg of xanthine per ml, 10 μg of thymidine per ml, 25 μg of adenine per ml, and 500 μg of glutamine per ml). Transformation frequencies were calculated by dividing the number of mycophenolic acid (MAXTA)-resistant colonies by the number of viable cells plated (from plating efficiency calculations).

Initially, DNA transfection conditions were varied to identify any differences between the mutant and strain AA8 in processing intact plasmid molecules. Figure 1B shows the data from an experiment in which EM9 or AA8 cells were exposed to pSV2-*gpt* DNA (2 μg per dish) for 4 to 16 h and placed under mycophenolic acid selection 48 h later. With increasing exposure time a linear increase in transformation frequency was observed in both strains. Transformation frequencies at different DNA amounts were then measured and were found to increase with DNA concentration similarly in both strains, as shown in Fig. 1C. Finally, the optimum expression time of the *gpt* gene after 16 h of DNA exposure was assessed (data not shown). Transformation frequencies measured from 1 to 8 days after exposure were remarkably similar for both the mutant and wild-type strains, indicating that the plasmid is stably integrated and expressed within 24 h and remains so for up to 8 days after DNA addition without mycophenolic acid selection. Taken together, these data indicate that under a variety of conditions, there were no differences between AA8 and EM9 in uptake and integration of pSV2-*gpt*.

The ability of AA8 and EM9 to repair double-strand breaks via ligation was then tested by introducing restriction cleavages within pSV2-*gpt*. Plasmid DNA was digested with *Hin*dIII, *Bgl*II, or *Kpn*I (which all produce cleavages which must be ligated for the gene to be expressed [Fig. 1A] [18, 19]), phenol extracted, and precipitated onto monolayers of EM9 or AA8 cells for 16 h. After the appropriate expression time, transformation to mycophenolic acid resistance was measured. Transformation frequencies from five experiments are shown in Table 1. *Kpn*I cleavage (which is within the coding sequence of the gene [18, 19]) reduced the transformation frequency to less than 1% that of the intact plasmid or of plasmid linearized with *Eco*RI (which does not require ligation for expression). *Kpn*I-cleaved plasmid was also treated with DNA ligase in vitro and was found to be functional in *Escherichia coli* (data not shown), indicating that the plasmid was still ligatable and not otherwise damaged by the digestion conditions. Cleavage with *Bgl*II or *Hin*dIII, which cut 80 and 200 base pairs upstream of the

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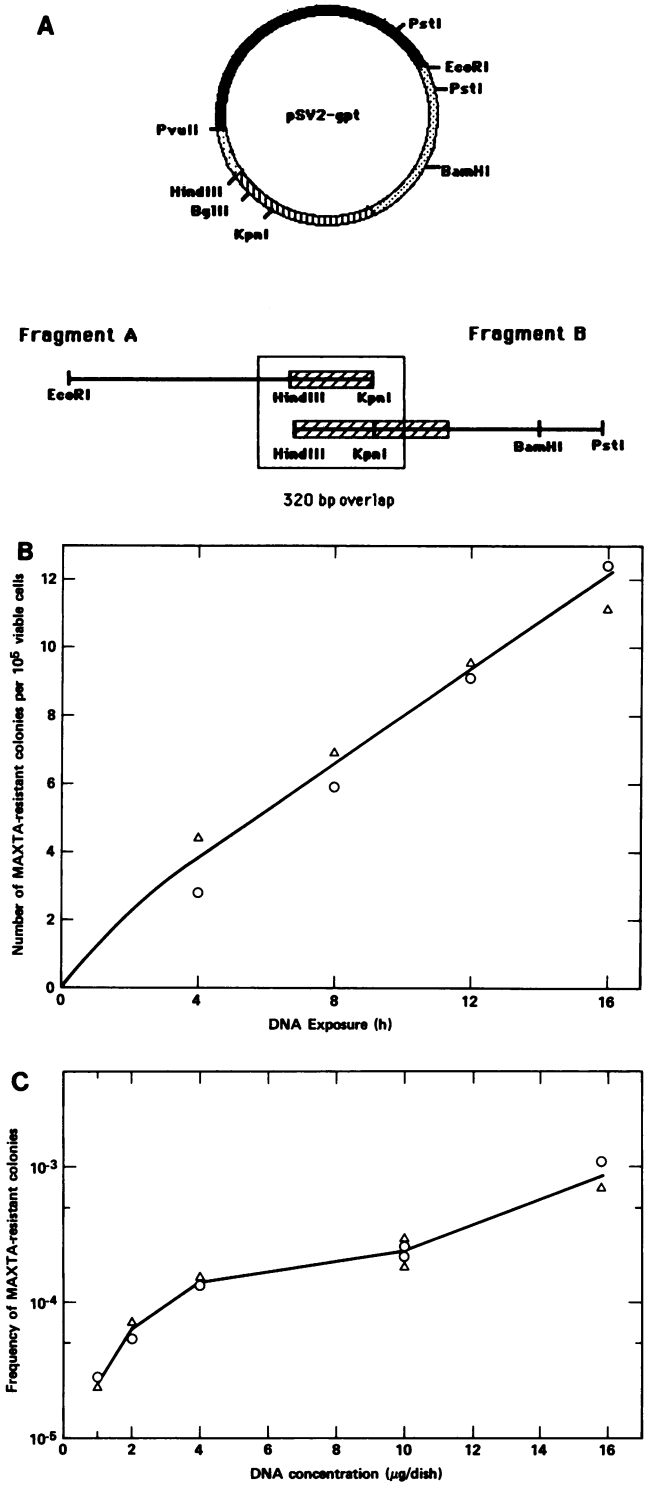

FIG. 1. (A) Diagram of circular plasmid pSV2-*gpt* (5.3 kilobases). The solid region represents pBR322 sequences; the dotted region indicates simian virus 40 sequences; and the cross-hatched region represents the *gpt* gene. Plasmid DNA was isolated from *E. coli* strain HB101 as previously described (16). Fragments A and B used to measure recombination frequencies in strains AA8 and EM9 are shown below the plasmid; the 320-base pair (bp) region of homology is indicated by the box; the cross-hatched region represents the *gpt* gene. (B) Dependence of transformation frequency on DNA exposure duration. DNA transfer was performed with calcium phosphate-precipitated DNA as described previously (35). Cell monolayers were exposed to pSV2-*gpt* DNA (2 μg/dish) for 4 to 16 h, allowed 48 h for expression, and then harvested and put under selection in MAXTA medium. Symbols: ○, AA8; △, EM9. (C) Dependence of transformation frequency on DNA concentration. DNA transfer was performed as described (35). Cell monolayers were exposed to increasing DNA concentration for 16 h, allowed 48 h for expression, and then harvested and put under selection in MAXTA medium. Transformation frequencies were corrected for plating efficiency. Symbols: ○, AA8; △, EM9.

start site, respectively, produced substantially higher transformation frequencies of 6 and 25%. Thus, although ligation is possible, modification of the ends of the molecules must occur during transfection since the frequency of transformation increases as the cut is distanced from the coding sequence. These results are in sharp contrast to the results of a previous study (5), in which the transformation frequencies of intact pSV2-*gpt* or *Kpn*I-, *Bgl*II-, or *Hin*dIII-cleaved pSV2-*gpt* were essentially the same in several human cell lines, possibly reflecting differences between human and hamster cells. Data from several other studies indicate that DNA can be heavily damaged during transfection (1, 3, 23). Also, extensive "nibbling" of transfected DNAs (on the order of several hundred base pairs) has been observed in hamster cells (C. Gasser, Ph.D. thesis, Stanford University, Stanford, Calif., 1985). The results from these studies support our finding that successful ligation and expression of a functional *gpt* gene from *Kpn*I-cut plasmid, where even the loss of 1 base pair would affect expression, are rare events in hamster cells.

To study the recombination capacity of AA8 and EM9, two fragments were isolated after digestion of pSV2-*gpt* with *Eco*RI and *Kpn*I (fragment A) or *Hin*dIII and *Pst*I (fragment B) (Fig. 1A). Fragment A contained a truncated form of the *gpt* gene, and fragment B contained the entire gene sequence but did not include the simian virus 40 promoter necessary for expression in mammalian cells. Neither fragment is capable of expression by itself. However, together the fragments contain a 320-base pair homologous region that upon recombination should produce a functional *gpt* gene. Table 2 summarizes the data of five experiments in which transformation frequencies were measured after transfection of the cells with equimolar quantities of the two fragments. Fragment A or B alone gave extremely low or undetected transformation frequencies. (The few colonies observed with fragment B may be explained by integration adjacent to a promoter, as previously observed [Gasser, Ph.D. thesis].) When the two fragments were coprecipitated onto AA8 cells, the transformation frequencies were almost equal (~91%) to those produced by equimolar amounts of the intact plasmid. On the other hand, strain EM9 consistently had transformation frequencies that were approximately threefold lower. Southern blot analysis of high-molecular-weight DNA isolated from two AA8 and three EM9 mycophenolic acid-resistant clones resulting from cotransfection with the two fragments was performed by using a 2.3 kilobase fragment purified from pSV2-*gpt* as a probe. As shown in Fig. 2, a 2.3-kilobase band, the correct size if recombination of fragments A and B had produced an intact *gpt* gene, was common among all five transformants. Thus, we concluded that the high transformation frequencies produced by coprecipitation of the two fragments were due to homologous recombination, which reconstructed a functional *gpt* gene, and that strain EM9 was only ~37% as efficient in recombination as strain AA8 (based on the average of the values given in Table 2).

TABLE 1. Transformation frequencies of enzyme-digested pSV2-*gpt*

Expt	Transformation frequencies									
	AA8 cells					EM9 cells				
	Uncut	*Eco*RI	*Kpn*I	*Bgl*II	*Hin*dIII	Uncut	*Eco*RI	*Kpn*I	*Bgl*II	*Hin*dIII
1[a,b]	2.0×10^{-5}	2.7×10^{-5}	1.8×10^{-6} (0.9)[c]			2.0×10^{-5}	2.9×10^{-5}	1.6×10^{-6} (0.8)		
2[a]	1.1×10^{-3}	7.7×10^{-4}	3.8×10^{-6} (0.3)			6.6×10^{-4}	6.5×10^{-4}	4.7×10^{-6} (0.7)		
3	4.3×10^{-4}		6.1×10^{-6} (1.0)		1.3×10^{-4} (30)					
4	4.8×10^{-4}		5.4×10^{-6} (1.0)	1.6×10^{-5} (3)	1.6×10^{-4} (33)	5.8×10^{-4}		5.4×10^{-6} (1.0)	4.7×10^{-5} (8.0)	1.4×10^{-4} (25)
5	8.4×10^{-4}		2.0×10^{-5} (2.5)	4.7×10^{-5} (8)	1.6×10^{-4} (19)	9.0×10^{-4}		2.0×10^{-5} (2.0)	3.2×10^{-5} (4.0)	1.8×10^{-4} (20)

[a] The transformation values (calculated as described in the text) for experiments 1 and 2 are the average values obtained from days 1, 2, and 4 of expression. The values for subsequent experiments were calculated from day 2 plating only.
[b] The DNA concentration was 2 μg/dish; in subsequent experiments a concentration of 16 μg/dish was used.
[c] The numbers in parentheses are the percentage of transformation relative to the frequency of the intact plasmid.

TABLE 2. Transformation frequencies from transfections with overlapping fragments of the *gpt* gene

Expt	Transformation frequencies								EM9/AA8 ratio[b]
	AA8 cells				EM9 cells				
	Intact	Fragment A[a]	Fragment B[a]	Fragments A and B	Intact	Fragment A	Fragment B	Fragments A and B	
1[c]	2.5×10^{-4}	$<1 \times 10^{-6}$	4×10^{-6}	1.8×10^{-4} (72)[d]	2.2×10^{-4}	$<1 \times 10^{-6}$	2×10^{-6}	9.0×10^{-5} (41)	0.50
2	2.7×10^{-4}	$<1 \times 10^{-6}$	2×10^{-6}	3.2×10^{-4} (119)	2.9×10^{-4}	$<1 \times 10^{-6}$	2×10^{-6}	1.2×10^{-4} (41)	0.34
3	1.4×10^{-4}	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	1.1×10^{-4} (79)	1.5×10^{-4}	$<1 \times 10^{-6}$	2×10^{-6}	4.0×10^{-5} (27)	0.34
4[c]	5.7×10^{-5}	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	5.4×10^{-5} (95)	7.8×10^{-5}	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	2.3×10^{-5} (29)	0.30
5					1.1×10^{-4}	$<1 \times 10^{-6}$	$<1 \times 10^{-6}$	3.4×10^{-5} (31)	

[a] Experiments 1 and 2 were done with 5 μg of each fragment; experiment 3 was done with 2 μg of each fragment; and experiments 4 and 5 were done with 1 μg of each fragment.
[b] Based on the percentages from the columns giving results for fragments A and B.
[c] The transformation frequencies for experiments 1 and 4 were calculated from the average transformation frequencies for platings from days 1, 2, and 4 of expression; the frequencies for the other three experiments were calculated from the average values for days 2 and 4 of expression.
[d] The numbers in parentheses are percentages, which were calculated by dividing the transformation frequencies of fragments A and B by the transformation frequencies of the intact plasmid.

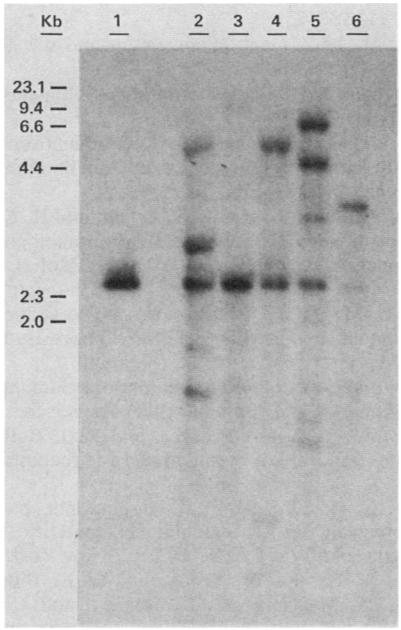

FIG. 2. Southern blot of high-molecular-weight DNA isolated from mycophenolic-acid resistant clones. High-molecular-weight DNA was isolated as previously described (16). For Southern transfer, 10 μg of DNA from each clone was digested with *Bam*HI and *Pvu*II and separated on a 0.8% agarose gel. The DNA was transferred from the gel to nitrocellulose paper as described previously (29). A fragment of plasmid pSV2-*gpt* containing the *gpt* gene was isolated after digestion with *Bam*HI and *Pvu*II (see Fig. 1) and radiolabeled with [^{32}P]dCTP (Amersham Corp.) by nick translation (7, 24). Hybridization of the filter with this probe was performed as described previously (7, 35). Lane 1 contained the purified *Pvu*II-*Bam*HI fragment (2.35 kilobases) as marker DNA; lanes 2 and 3 contained MAXTA-resistant clones of AA8; and lanes 4 through 6 contained MAXTA-resistant clones of EM9.

These frequencies again differ quantitatively from those of Cox et al. (5), who used similar fragments. The frequencies of Cox et al., obtained with two human cell lines, were 10-fold lower than those measured for AA8 and may again reflect differences between human and hamster cells. Although the AA8 frequencies may appear to be surprisingly high, it should be noted that we used linearized fragments with double-strand breaks adjacent to the region of homology. The dramatic effect of double-strand breaks on recombination frequencies is well established (2, 12, 15, 28), and

one model proposes that a double-strand break is required for initiation of recombination (31). Therefore, the frequencies measured with AA8 are in accordance with those published previously for other cell lines and other vectors.

In conclusion, the processing of foreign DNA through homologous recombination was less efficient in mutant EM9, suggesting that the biochemical defect underlying the EM9 mutation may be specifically involved in recombination. Because integration and expression of intact and linearized plasmid was normal in EM9 cells under a variety of conditions, our data imply that this defective aspect of homologous recombination may not be involved with integration or ligation. The relationship of this finding to the high sister chromatid exchange frequencies found in the mutant is unclear at this time, although it is known that the sister chromatid exchanges in EM9 appear to result mainly from the incorporated bromodeoxyuridine used in their detection (20) and that defects in DNA ligase (4), poly(ADP-ribose) metabolism (10), or apurinic/apyrimidimic endonuclease (13) are not involved. Furthermore, our results suggest that the enzymatic machinery by which mammalian cells process exogenous DNA overlaps with that which maintains genomic DNA. Therefore, further studies of this type with EM9 or other available DNA repair mutants (8, 11, 33, 34; see reference 32 for a review) should be useful in elucidating the metabolism of genomic DNA.

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LITERATURE CITED

1. **Ashman, C. R., and R. L. Davidson.** 1984. High spontaneous mutation frequency in shuttle sequences recovered from mammalian cellular DNA. Mol. Cell. Biol. **4**:2266–2272.
2. **Brenner, D. A., A. C. Smigocki, and R. D. Camerini-Otero.** 1985. Effect of insertions, deletions, and double-strand breaks on homologous recombination in mouse L cells. Mol. Cell. Biol. **5**:684–691.
3. **Calos, M. P., J. S. Lebowski, and M. R. Botchan.** 1983. High mutation frequency in DNA transfected into mammalian cells. Proc. Natl. Acad. Sci. USA **80**:3015–3019.
4. **Chan, J. Y. H., L. H. Thompson, and F. F. Becker.** 1984. DNA ligase activities appear normal in the CHO mutant EM9. Mutat. Res. **131**:209–214.
5. **Cox, R., W. K. Masson, P. G. Debenham, and M. B. T. Webb.** 1984. The use of recombinant DNA plasmids for the determination of DNA-repair and recombination in cultured mammalian cells. Br. J. of Cancer **49**(Suppl. 6):67–72.
6. **De Saint Vincent, B. R., and G. M. Wahl.** 1983. Homologous recombination in mammalian cells mediated formation of a functional gene from two overlapping gene fragments. Proc. Natl. Acad. Sci. USA **80**:2002–2006.
7. **Fuscoe, J. C., R. G. Fenwick, Jr., D. H. Ledbetter, and C. T. Caskey.** 1983. Deletion and amplication of the HGPRT locus in Chinese hamster cells. Mol. Cell. Biol. **3**:1086–1096.
8. **Giaccia, A., R. Weinstein, J. Hu, and T. Stamato.** 1985. Cell-cycle dependent repair of double-strand breaks in a γ-ray sensitive Chinese hamster cell. Somatic Cell Mol. Genet. **11**:485–491.
9. **Hoy, C. A., E. P. Salazar, and L. H. Thompson.** 1984. Rapid detection of DNA damaging agents using repair-deficient CHO cells. Mutat. Res. **130**:321–332.
10. **Ikejima, M., D. Bohannon, D. M. Gill, and L. H. Thompson.** 1984. Poly(ADP-ribose) metabolism appears normal in EM9, a mutagen-sensitive mutant of CHO cells. Mutat. Res. **128**: 213–220.
11. **Kemp, L. M., S. G. Sedgwick, and P. A. Jeggo.** 1984. X-ray sensitive mutants of Chinese hamster ovary cells defective in double-strand break rejoining. Mutat. Res. **132**:189–196.
12. **Kucherlapati, R. S., E. M. Ewes, K.-Y. Song, B. S. Morse, and O. Smithies.** 1984. Homologous recombination between plasmids in mammalian cells can be enhanced by treatment of input DNA. Proc. Natl. Acad. Sci. USA **81**:3153–3157.
13. **LaBelle, M., S. Linn, and L. H. Thompson.** 1984. Apurinic /apyridimic endonuclease activities appear normal in the CHO-cell ethylmethane sulfonate mutant, EM9. Mutat. Res. **141**:4–44.
14. **Latt, S. A.** 1981. Sister chromatid exchange formation. Annu. Rev. Genet. **15**:11–56.
15. **Lin, F.-W., K. Sperle, and N. Sternberg.** 1984. Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4**:1020–1034.
16. **Maniatis, T., E. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Miller, C. K., and H. M. Temin.** 1983. High efficiency ligation and recombination of DNA fragments by vertebrate cells. Science **220**:606–609.
18. **Mulligan, R. C., and P. Berg.** 1981. Factors governing the expression of a bacterial gene in mammalian cells. Mol. Cell. Biol. **1**:449–459.
19. **Mulligan, R. C., and P. Berg.** 1981. Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyltransferase. Proc. Natl. Acad. Sci. USA **78**:2072–2076.
20. **Pinkel, D., L. H. Thompson, J. W. Gray, and M. Vanderlaan.** 1985. Measurement of sister chromatid exchange at very low bromodeoxyuridine substitution levels using a monoclonal antibody in Chinese hamster ovary cells. Cancer Res. **45**: 5795–5798.
21. **Pomeranz, B. J., M. Naujokas, and J. A. Hassell.** 1983. Homologous recombination between transfected DNAs. Mol. Cell. Biol. **3**:1680–1685.
22. **Radding, C. M.** 1982. Homologous pairing and strand exchange in genetic recombination. Annu. Rev. Genet. **16**:405–437.
23. **Razzaque, A., H. Mizusawa, and M. M. Sewidman.** 1983. Rearrangement and mutagenesis of a shuttle vector plasmid after passage in mammalian cells. Proc. Natl. Acad. Sci. USA **80**:3010–3014.
24. **Rigby, P. W. J., M. Diekmann, C. Rhodes, and P. Berg.** 1977. Labeling *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
25. **Robertson, M.** 1982. Gene arrangement and the generation of diversity. Nature (London) **297**:184–186.
26. **Shimizu, A., and T. Honjo.** 1984. Immunoglobin class switching. Cell **36**:801–803.
27. **Small, J., and G. Scangos.** 1983. Recombination during gene transfer into mouse cells can restore the function of deleted genes. Science **219**:174–176.
28. **Song, K., L. Chikuri, S. Rauth, S. Erlich, and R. Kucherlapati.** 1985. Effect of double-strand breaks on homologous recombination in mammalian cells and extracts. Mol. Cell. Biol. **5**: 3331–3336.
29. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
30. **Stahl, F. W.** 1979. Genetic recombination: thinking about it in phage and fungi. W. H. Freeman, San Francisco.
31. **Szostak, J. W., T. L. Orr-Weaver, and R. J. Rothstein.** 1983. The double strand break repair model for recombination. Cell **33**:25–35.
32. **Thompson, L. H.** 1985. DNA repair mutants, p. 641–667. *In* M. M. Gotesman (ed.), Molecular cell genetics: the Chinese hamster cell. John Wiley & Sons, Inc., New York.
33. **Thompson, L. H., K. W. Brookman, L. E. Dillehay, A. V. Carrano, J. A. Mazrimas, C. L. Mooney, and J. A. Minkler.** 1982. A CHO-cell strain having hypersensitivity to mutagens, a

defect in strand-break repair, and extraordinary baseline frequency of sister-chromatid exchange. Mutat. Res. **95**:427–440.

34. **Thompson, L. H., K. W. Brookman, L. E. Dillehay, C. L. Mooney, and A. V. Carrano.** 1982. Hypersensitivity to mutation and sister-chromatid exchange induction in CHO cell mutants defective in incising DNA containing UV lesions. Somatic Cell Genet. **8**:759–773.
35. **Thompson, L. H., K. W. Brookman, J. L. Minkler, J. C. Fuscoe, K. A. Henning, and A. V. Carrano.** 1985. DNA-mediated transfer of a human DNA repair gene that controls sister chromatid exchange. Mol. Cell. Biol. **5**:881–884.
36. **Thompson, L. H., S. Fong, and K. Brookman.** 1980. Validation of conditions for efficient detection of HPRT and APRT mutations in suspension-cultured Chinese hamster ovary cells. Mutat. Res. **74**:21–36.
37. **Thompson, L. H., J. S. Rubin, J. E. Cleaver, G. F. Whitmore, and K. Brookman.** 1980. A screening method for isolating DNA repair deficient mutants of CHO cells. Somatic Cell Genet. **6**:391–405.
38. **Upcroft, P., B. Carter, and C. Kidson.** 1980. Analysis of recombination in mammalian cells using SV40 genomic segments having homologous overlapping termini. Nucleic Acids Res. **8**:2725–2735.
39. **Volkert, F. C., and C. S. H. Young.** 1983. The genetic analysis of recombination using adenovirus overlapping terminal DNA fragments. Virology **125**:175–193.
40. **Wake, C. T., and J. H. Wilson.** 1979. Simian virus 40 recombinants are produced at high frequency during infection with genetically mixed oligomeric DNA. Proc. Natl. Acad. Sci. USA **76**:2876–2880.
41. **Yunis, J. J.** 1983. The chromosomal basis of human neoplasia. Science **221**:227–236.