
Recombination events during integration of transfected DNA into normal human cells

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ABSTRACT

The mechanisms of recombination responsible for random integration of transfected DNA into the genome of normal human cells have been investigated by analysis of plasmid-cell DNA junctions. Cell clones containing integrated plasmid sequences were selected by morphological transformation of primary human fibroblasts after transfection with a plasmid containing simian virus 40 sequences. Nucleotide sequence analysis of the plasmid-cell DNA junctions was performed on cloned DNA fragments containing the integration sites from two of these cell clones. Polymerase chain reaction was then performed with human cell DNA from primary fibroblasts to isolate the cell DNA from the same sites before plasmid integration. Comparison of the sequences at the plasmid-cell DNA junctions with those of both the original plasmid and the cell DNA demonstrated short sequence similarities and additional nucleotides, typical of nonhomologous recombination. Evidence of short deletions in the cell DNA at the plasmid integration sites suggests that integration occurred by a mechanism similar to that used for repair of spontaneous or gamma ray-induced strand breaks. Plasmid integration occurred within nonrepetitive cell DNA with no major rearrangements, although rearrangements of the cell DNA at the integration site occurred in one of the clones after integration.

INTRODUCTION

Transfection of DNA into mammalian cells has become an increasingly important procedure. Little information is available, however, about the actual events involved in the integration process. Although techniques are now available for selection of integrations into specific locations (1, 2), integration appears to be predominantly random (3-6). (This conclusion is based mostly on circumstantial evidence.) Several studies have also demonstrated that some integration sites can be highly unstable, leading to rearrangements in the integrated plasmid as well as in the surrounding cell DNA (7-10). Optimizing the efficiency of targeted integration, therefore, requires additional information

on what factors influence homologous integration and on how integrated foreign sequences affect chromosome stability.

To learn more about the integration process, one study analyzed both plasmid-cell DNA junctions at three random integration sites in a mouse cell line (6). Although the original sites before integration were not isolated for comparison, the results suggested that little if any sequence similarity was necessary for integration. Integration occurred primarily within regions of repetitive DNA and resulted in major rearrangements in the cell DNA. It is therefore often assumed that most integrations are associated with major rearrangements in the cell DNA; however, one study has demonstrated that integration of viral DNA can occur directly without additional rearrangements (11).

In the current study we have conducted a similar analysis of random plasmid integration sites in primary human fibroblasts. Selection of clones containing integrated plasmids was accomplished by isolation of morphologically transformed foci that resulted from integration of origin-of-replication-defective SV40 sequences (12). In addition to analyzing the plasmid-cell DNA junctions, we also analyzed the cell DNA at these sites before integration for a direct comparison. The results therefore provide information on both the nature of the sequences involved in the recombination and the consequences of integration on cell DNA.

MATERIALS AND METHODS

Cell culture. The normal primary human skin fibroblast strain HS-27 used in these studies was obtained from the Cell Culture Facility, University of California, San Francisco. Cell cultures were maintained in minimal essential medium containing either 10% (transformed lines) or 15% (primary fibroblasts) fetal calf serum. The cells were maintained at 37°C in a humidified incubator containing 10% CO₂.

Cell transformation. Cell transformation was performed with the *Bgl*III-linearized plasmid pLR309 (12), which contains origin-of-replication-defective SV40 sequences (13). After calcium phosphate-mediated transfection of the plasmid (12), morphologically transformed foci were selected as described previously (12).

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DNA cloning. Integrated plasmid sequences and surrounding cell DNA were isolated with the use of bacteriophage cloning vectors as described previously (12). Portions of the cloned DNA to be used for sequencing or as probes for hybridization were further cloned into the Bluescript plasmid (Stratagene).

Nucleotide sequence analysis. Nucleotide sequence analysis was conducted as described previously (10), according to the method of Sanger *et al.* (14). Sequencing was performed with Sequenase (U.S. Biochemical Corp.) with the use of plasmid minipreps (15) purified on Sephacryl S-400 columns (Pharmacia). The sequences for the *neo* gene (16), SV40 (17), and pBR322 (18) have been reported previously.

Polymerase chain reaction. Primers (22 nucleotides) corresponding to the cellular DNA sequences adjacent to the integration sites were synthesized by the Biomolecular Resource Center, University of California, San Francisco. Polymerase chain reaction was performed as described previously (19), with 1 μ g of HS-27 cell DNA that had been denatured by boiling for 10 min, an annealing temperature of 60°C, and 40 cycles. The polymerase chain reaction products (651 bp for LM205, 202 bp for LM216) were digested with restriction enzymes and cloned into Bluescript (Stratagene) for nucleotide sequencing. Several products were analyzed to eliminate possible errors by the Taq polymerase.

Southern and slot blot analysis. Agarose gel electrophoresis, blotting, and hybridization with radioactive or biotin-labeled probes have been described previously (12, 20).

RESULTS

Mapping of integration sites

Two permanent human cell lines (LM205, LM216) established by DNA transfection of primary human fibroblasts with the

linearized pLR309 plasmid (Fig. 1A) contain 3 and 1 plasmid integration sites, respectively (12). *Bgl*III restriction fragments containing the integrated plasmid sequences and surrounding cell DNA from both LM205 (12) and LM216 (this study) were cloned into bacteriophage cloning vectors. Mapping of restriction enzyme cleavage sites, hybridization with specific probes, and partial nucleotide sequence analysis were used to determine the structure of the integrated plasmid sequences. The cloned integration site from LM205 (Fig. 1B) contains 10.4 kb of plasmid DNA, with approximately 2.2 kb of cell DNA on one side and 1.8 kb of cell DNA on the other side. The cloned integration site from LM216 (Fig. 1C) contains 9.6 kb of plasmid DNA, with 1.5 kb of cell DNA on one side and 5.5 kb of cell DNA on the other side. The plasmid sequences in LM205 consist of a single, nearly intact copy of the pLR309 plasmid, whereas LM216 contains several plasmid fragments integrated in tandem. These tandem fragments may have been formed before integration; however, many rearrangements of integrated plasmid sequences also occur after integration (12, 20).

Nucleotide sequences at plasmid-cell DNA junctions

Integration in cell clone LM205 occurred by recombination between cell DNA and *neo* gene sequences at one terminus, and between cell DNA and pBR322 sequences at the other (Fig. 1B). In cell clone LM216, integration resulted from recombination between cell DNA and pBR322 sequences at one terminus, and between cell DNA and SV40 sequences at the other (Fig. 1C). To determine the structure of the DNA directly involved in the integration, we sequenced through the plasmid-cell DNA junctions in both cell clones (Fig. 2). Although this procedure revealed the plasmid sequences at the site of the junction with the cell DNA, it did not tell us whether any sequence similarity was involved, or what effect integration had on the cell DNA. Oligonucleotide primers were therefore synthesized from the region of cell DNA to either side of the integration sites, and polymerase chain reaction was performed with human cell DNA

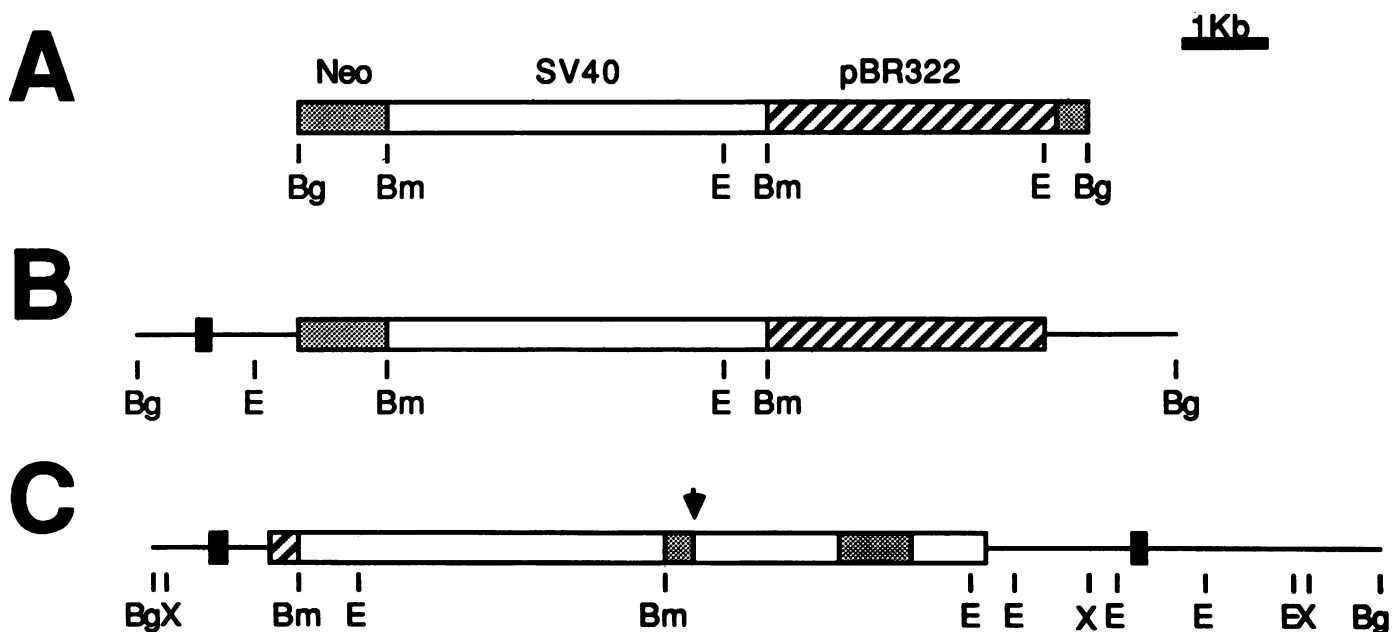


Figure 1. Structure of the linearized pLR309 plasmid (A) is compared with the integrated plasmid sequences isolated from cell clones LM205 (B) and LM216 (C). The *neo* gene, SV40 sequences, and pBR322 sequences are indicated, and adjacent cell sequences are represented by thin solid lines. The approximate locations of *Alu* sequences are shown by black boxes, and restriction sites are indicated for *Bam*HI (Bm), *Bgl*III (Bg), *Eco*RI (E), and *Xba*I (X). The vertical arrow shows an internal recombination site that was also analyzed.

from primary fibroblasts to isolate the cell DNA that existed in these regions before integration. The resulting DNA fragments were then sequenced and compared with the cell-plasmid junctions and plasmid DNA sequences (Fig. 2).

The results with LM205 (Fig. 2A) demonstrated that upon integration 12 bp were lost from the end of the plasmid with the *neo* gene, and 347 bp were lost from the pBR322 end. A 311-bp deletion also occurred internally within the pBR322 sequences 9 bp from the plasmid-cell DNA junction; there was no sequence similarity between the sequences involved in the recombination, and a single C-G base pair was added (data not shown). Comparison of the integration site in LM205 with the cell DNA before integration (Fig. 2A) demonstrated no similarity between the integrated portion of the plasmid and cell DNA at either junction, with C-G base pairs inserted at both junctions. A 9-bp deletion occurred in the cell DNA at the integration site. This deletion included part of a *Bgl*III restriction site. Integration may therefore have involved identity between the 4-bp single-stranded ends of the plasmid that were generated by linearization with *Bgl*III, although the ends of the plasmid were lost during integration.

The results with LM216 (Fig. 2B) demonstrated 1 bp of similarity between pBR322 sequences and cell DNA at one end of the integrated plasmid, and 6 bp of similarity between SV40 sequences and cell DNA at the other end. Integration occurred within an A-T-rich region of cell DNA (31 of 32 bp) and resulted in a loss of 12 bp of cell DNA at the integration site. One of the plasmid-cell DNA junctions occurred within a poly-T region. The length of this region in the cell DNA varied among different

polymerase chain reaction products; three products had 12 consecutive thymidines (Fig. 2B), two products had 11, and one product had 9, apparently owing to errors with the Taq polymerase.

The nucleotide sequence of one of the internal recombination sites within the plasmid sequences in LM216 was also determined (Fig. 2C). Recombination occurred between the *neo* gene and the SV40 sequences. There was no sequence similarity at the site of the junction, and two additional A-T base pairs were inserted. A 13-bp inversion also occurred within the *neo* gene sequences at the recombination site. The site of the inversion corresponded with 4 C-G base pairs found at either end, and therefore may have involved the similarity between these sites. Whether this junction and inversion were formed before or after integration is not known.

Nature of the cellular sequences at the integration site

The fact that the polymerase chain reaction products of the DNA at the integration sites in LM205 and LM216 were unique fragments suggested that these integrations occurred in nonrepetitive DNA. To confirm this observation, the cellular DNA sequences cloned from the integration sites in LM205 and LM216 were used as probes and hybridized to DNA from a separate cell line. The 1.8 kb of LM205 cell DNA to the right of the integration site (Fig. 1) hybridized to single nonrepetitive bands on Southern blots; however, extensive heterogeneous hybridization indicated that interspersed repetitive DNA was located within the 2.2 kb of cell DNA to the left of the integration site (ref. 20; Table 1). A DNA probe consisting of the first 1215

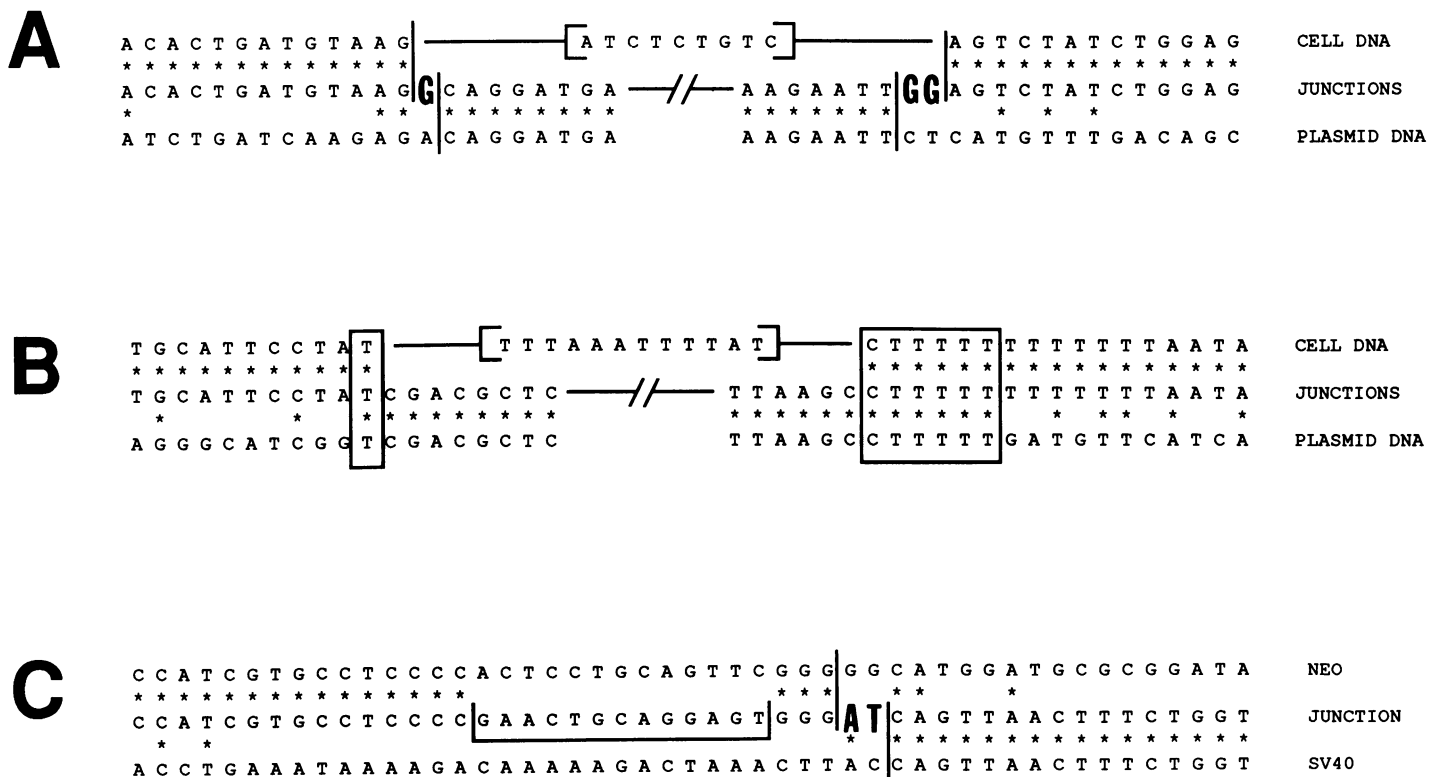


Figure 2. Nucleotide sequence analysis of the cell-plasmid DNA junctions at the integration sites in LM205 (A) and LM216 (B), and a site of recombination within the plasmid sequences in LM216 (C). Comparison is made with the original cell DNA and *neo* (16), SV40 (17), or pBR322 (18) sequences found in the plasmid. Similar nucleotides are indicated by stars, and junctions between the plasmid and cell DNA at the integration site are shown by vertical lines. Similarities between the sequences at the junctions are enclosed in boxes, and nucleotides added during integration are shown in bold type. The nucleotides deleted from the cell DNA are enclosed in brackets, and the inverted region is underlined.

Table 1. Absence of major rearrangements in the cell DNA during plasmid integration

Cell clone	Enzyme	After integration	Restriction fragment size	
			Before integration	Expected if no other rearrangement ^a
LM205	<i>Bgl</i> II	14 ^b	2.2 + 1.8 ^c	4
	<i>Sst</i> I	22 ^b	12 ^d	12
	<i>Xba</i> I	22 ^b	12 ^e	12
LM216	<i>Bgl</i> II	17 ^{b, f}	7.6 ^e	7.4
	<i>Sst</i> I	15.5 ^b	6.4 ^e	5.9
	<i>Xba</i> I	12.5 ^f	3.1 ^e	2.9

^aSubtract size of integrated plasmid sequence (10.4 kb, LM205; 9.6 kb, LM216) from size of fragment after integration.

^bData from ref. 12.

^c2.2-kb fragment observed by hybridization with cell DNA probe from the left of the integration site (unpublished observation). 1.8-kb fragment observed by hybridization with cell DNA probe from the right of the integration site (20).

^dData from ref. 20.

^eUnpublished observations.

^fSee Fig. 1.

bp of DNA to the left gave only single bands by Southern blot analysis (data not shown), suggesting that an *Alu* sequence shown by sequence analysis to be located 1236 to 1450 bp from the integration site is the repetitive sequence closest to this integration site. Slot blot analysis (Fig. 3) confirmed these results. Probes consisting of the 1.8 kb of cell DNA to the right of the integration site or 778 bp of cell DNA to the left of the integration site gave minimal hybridization to DNA from primary human fibroblasts. In contrast, a probe containing the cell DNA 779 bp to 2.2 kbp to the left of the integration site gave extensive hybridization. An additional probe containing cell DNA 1380 bp to 2.2 kbp to the left of the integration site, which contained only 70 bp of the *Alu* sequence in this region, showed an intermediate level of hybridization.

The 1.5 kb of LM216 cell DNA closest to the right of the integration site (Fig. 1) gave single bands when used as a probe for Southern blot analysis (Table 1), indicating that integration in this cell clone also occurred within nonrepetitive cell DNA. Hybridization with the rest of the cell sequences cloned from LM216 as a probe, however, demonstrated interspersed repetitive sequences in the region of the integration site (data not shown). Southern blot analysis with a probe containing *Alu* sequences (Fig. 4) revealed several *Alu* sequences in the cell DNA adjacent to the integration site in LM216. The *Alu* sequences in the cell DNA to the right of the integration site were at least 2 kb from the integration site (Fig. 1C), as shown by hybridization to restriction fragments (2.3 kb for *Xba*I, 1.2 kb for *Eco*RI or *Eco*RI plus *Xba*I) that contained this region. *Alu* sequences were also detected within restriction fragments containing the 1.5 kb of cell DNA to the left of the LM216 integration site (Fig. 1C). These fragments were 26 kb for *Eco*RI (which includes bacteriophage DNA), 12.5 kb for *Xba*I (also seen as a lighter 9-kb band owing to deletions in the bacteriophage by homologous recombination), and 2.5 kb for *Eco*RI plus *Xba*I. The exact location of *Alu* sequences within this region has not been determined; however, they must be at least 85 bp away from the integration site, as demonstrated by nucleotide sequence analysis of plasmid-cell DNA junctions (Fig. 2). Whether there are other repetitive sequences in this region is not known, but sequences immediately surrounding the integration site show no homology with known repetitive elements.

Absence of major rearrangements in the cell DNA during integration

Restriction site patterns in the cell DNA before integration of the plasmid sequences were used to determine whether major rearrangements occurred in the cell DNA during integration. For this purpose, nonrepetitive sequences isolated from the integration sites in LM205 (ref. 20; Table 1) and LM216 (Table 1) were used as probes for Southern blot analysis of DNA from cells lacking plasmids at these integration sites. As shown in Table 1, the results for both LM205 and LM216 are consistent with the absence of major rearrangements of cell DNA in the region of the integration sites. Restriction enzyme digestion of DNA from other cell lines gave fragments of the sizes expected if the size of the integrated plasmid sequences was subtracted from the fragment sizes obtained with LM205 or LM216 (Table 1). The only exception was the *Bgl*II fragment in LM205, in which a *Bgl*II site was lost during integration (Fig. 2A).

DISCUSSION

Nucleotide sequence analysis of DNA from the integration sites in LM205 and LM216 showed that little or no sequence similarity is required for integration of foreign sequences into normal human cells. Short sequence similarities of 1 and 6 bp were observed at the cell-plasmid junctions at one integration site (LM205), and there were no similarities at the other integration site (LM216). The absence of sequence requirements is consistent with the apparently random integration of viral (21, 22) and transfected (3–6) DNA. Short sequence similarities at recombination junctions are typical of nonhomologous recombination between transfected DNA sequences (23, 24), as well as in cell DNA after spontaneous or gamma ray-induced DNA damage (25), and have been demonstrated to result from ligation of free ends (24, 25). The integration in LM205 occurred within a *Bgl*II site, which is relatively infrequent in human cell DNA. Recombination may therefore have involved the single-stranded ends of the plasmid DNA that resulted from linearization with *Bgl*II. If so, the end of the plasmid must have been lost after synapsis with the cell DNA.

The plasmid insertions were accompanied by short deletions within the cellular DNA: a 9-bp deletion in LM205 and a 12-bp

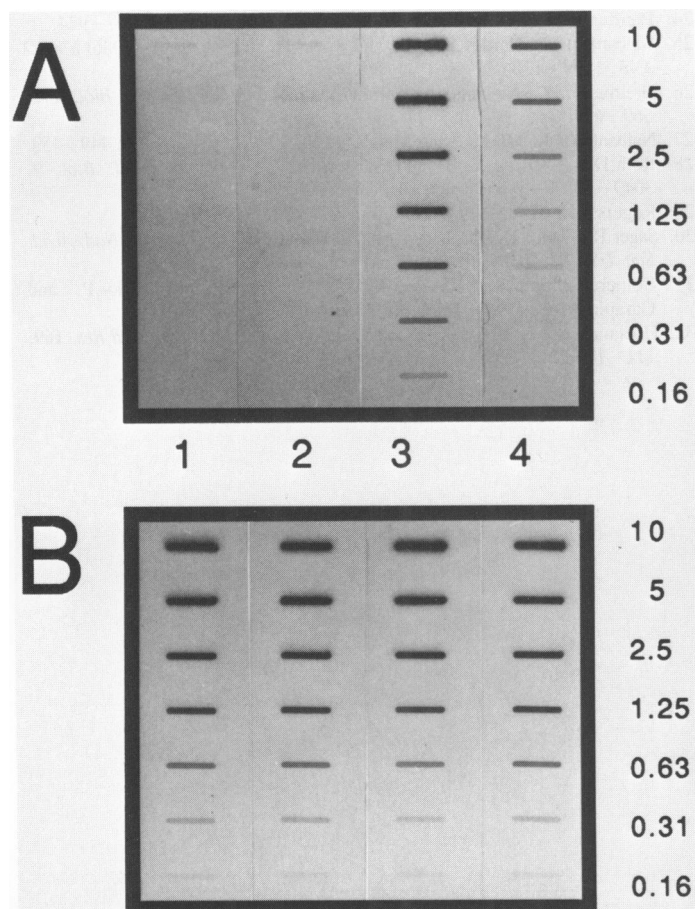


Figure 3. Slot blot analysis to determine the approximate position of repetitive sequences near the integration site in LM205. (A) Filters with various amounts of human DNA (10–0.16 μg) were hybridized with a probe containing the 1.8 kb of cell DNA to the right (Fig. 1A) of the integration site (lane 1); the 778 bp of cell DNA extending to the *Eco*RI site to the left of the integration site (lane 2); the 1.4 kb of cell DNA beginning after the *Eco*RI site to the left of the integration site (lane 3); and cell DNA beginning at 1380 bp to the left of the integration site and extending to 2.2 kbp from the integration site (lane 4), which contains 70 bp of an *Alu* sequence located 1183 to 1495 bp from the integration site. All probes were labeled with [α - ^{32}P]dCTP to similar specific activities. (B) The same blots shown in (A) but hybridized with ^{35}S -labeled probe consisting of the 2.2 kb of cell DNA to the left of the integration site to demonstrate that similar amounts of cell DNA were present on the filter.

deletion in LM216 (Fig. 2). Short deletions of this type are typical of those observed in spontaneous or gamma ray-induced mutations in mammalian cells (25, 26), which occasionally also involve insertion of cellular DNA (27). It is tempting to speculate, therefore, that the integration occurs by simple end ligation of the plasmid sequences into spontaneous breaks in the cell DNA, as proposed previously (2). Rearrangements and deletions in the plasmid DNA would therefore have occurred before integration, which is consistent with the absence of similar rearrangements within the cellular DNA.

A further indication of plasmid insertion at break sites in the cellular DNA is the presence of additional nucleotides at the recombination junctions. The insertion of additional nucleotides is often a feature of junctions formed during nonhomologous recombination (23, 28). In clone LM205, additional C-G base pairs were observed at both plasmid-cell DNA junctions as well as within a deletion in the pBR322 sequence (Fig. 2). Why there was an apparent preference for C-G base pairs, and whether these

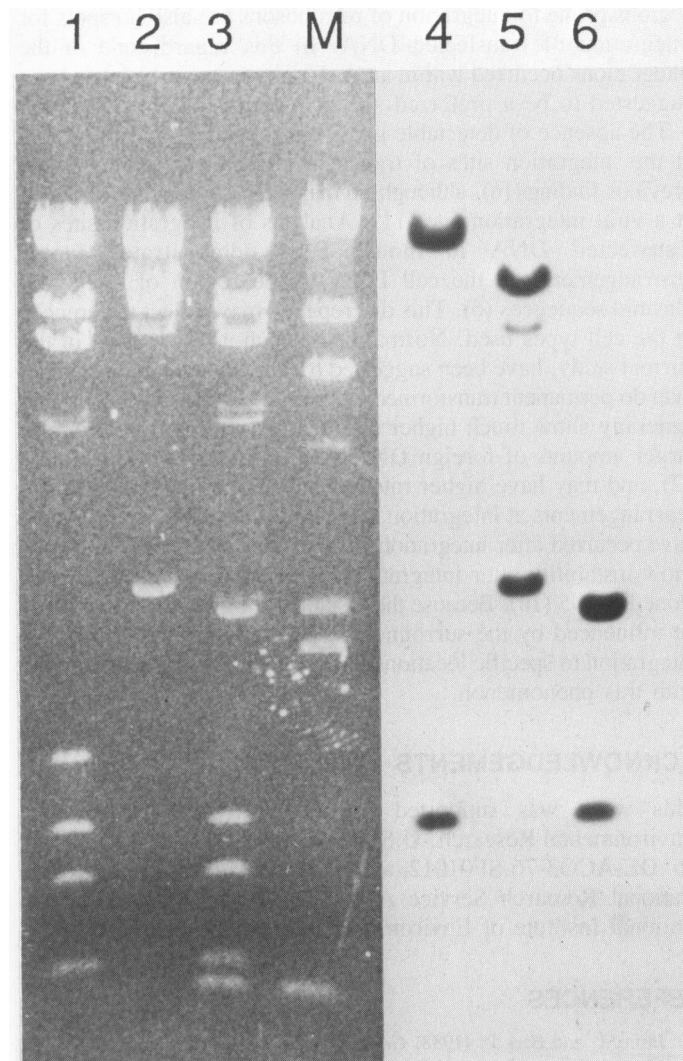


Figure 4. Southern blot analysis of *Alu* repetitive sequences found within the cell DNA cloned from LM216. The bacteriophage vector containing the cloned DNA was digested with *Eco*RI (lanes 1, 4), *Xba*I (lanes 2, 5), or *Eco*RI and *Xba*I (lanes 3, 6), separated on agarose gels (lanes 1–3), and Southern blot analysis performed (lanes 4–6) using a probe consisting of the 2.2-kb fragment of cell DNA containing the *Alu* sequence isolated from the integration site in LM205. Molecular weight markers (M) consisting of the 23.1-kb, 9.4-kb, 6.7-kb, 4.4-kb, 2.3-kb, 2.0-kb and 0.57-kb *Hind*III restriction fragments of lambda bacteriophage DNA

nucleotides appeared before or during integration, we do not know.

Although spontaneous or ionizing radiation-induced integration of cellular DNA fragments has been reported to occur within nonrepetitive gene sequences (27), a previous study in mouse cells suggested that repetitive sequences are preferred sites for random integration of transfected DNA (6). In the present study integration occurred within nonrepetitive DNA in both LM205 and LM216, and therefore repetitive sequences are not required for integration in normal human cells. Interspersed repetitive sequences were observed in the general region of the integration site in both clones; however, in cell clone LM205 these sequences were more than 1000 bp away. Because human cell DNA contains several hundred thousand interspersed *Alu* retroposons (29), integration near *Alu* retroposons would be expected even if integration were totally random. However, it is possible that

regions prone to integration of retroposons are also hotspots for integration of transfected DNA. In this regard, one of the integrations occurred within an A-T-rich region, which has been suggested to be a preferred site of retroposon insertion (29).

The absence of detectable gross rearrangements in cell DNA at the integration sites of transfected DNA also differs from previous findings (6), although no major rearrangements occurred at a viral integration site (11). Analysis of integration sites of transfected DNA in mouse cells demonstrated major rearrangements in the cell DNA in the region of integrated plasmid sequences (6). This discrepancy may reflect differences in the cell types used. Normal cells, such as those used in the current study, have been suggested to have more stable genomes than do permanent transformed clones (30). Rodent cell lines also generally show much higher transfection rates and incorporate larger amounts of foreign DNA than do human cell lines (31, 32), and may have higher rates of recombination. Many of the rearrangements at integration sites in mouse cells may therefore have occurred after integration. Integration sequences commonly show instability after integration (7–9), as has been shown in clone LM205 (10). Because the instability of integration sites may be influenced by the surrounding cell DNA (7–10), targeting integration to specific locations may prevent problems associated with this phenomenon.

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REFERENCES

- Jasin, M. and Berg, P. (1988) *Genes Devel.* **2**, 1353–1363.
- Mansour, S.L., Thomas, K.R. and Capecchi, M.R. (1988) *Nature* **336**, 348–352.
- Pellicer, A., Wigler, M., Axel, R. and Silverstein, S. (1978) *Cell* **14**, 133–141.
- Hsiung, N., Warrick, H., deRiel, J.K., Tuan, D., Forget, B.G., Skoultchi, A. and Kucherlapati, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4852–4856.
- Robins, D.M., Ripley, S., Henderson, A.S. and Axel, R. (1981) *Cell* **23**, 29–39.
- Kato, S., Anderson, R.A. and Camerini-Otero, R.D. (1986) *Mol. Cell. Biol.* **6**, 1787–1795.
- Perucho, M., Hanahan, D. and Wigler, M. (1980) *Cell* **22**, 309–317.
- Butner, K.A. and Lo, C.W. (1986) *J. Mol. Biol.* **187**, 547–556.
- Heartlein, M.W., Knoll, J.H.M. and Latt, S.A. (1988) *Mol. Cell. Biol.* **8**, 3611–3618.
- Murnane, J.P. and Young, B.R. (1989) *Gene* **84**, 201–205.
- Gahlmann, R. and Doerfler, W. (1983) *Nucl. Acids Res.* **11**, 7347–7361.
- Murnane, J.P. (1986) *Mol. Cell. Biol.* **6**, 549–558.
- Small, M.B., Gluzman, Y. and Ozer, H.L. (1982) *Nature* **296**, 671–672.
- Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
- Birnboim, H.C. and Doly, J. (1979) *Nucl. Acids Res.* **7**, 1513–1523.
- Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B. and Schaller, H. (1982) *Gene* **19**, 327–336.
- Reddy, V.B., Thimmappaya, B., Dhar, R., Subramanian, K.N., Zain, B.S., Pan, J., Ghosh, P.K., Celma, M.L. and Weissman, S.M. (1978) *Science* **200**, 494–502.
- Sutcliffe, J.G. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 77–90.
- Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) *Science* **239**, 487–491.
- Murnane, J.P. and Yezzi, M.J. (1989) *Som. Cell Mol. Genet.* **14**, 273–286.
- Sambrook, J., Greene, R., Stringer, J., Mitchison, T., Hu, S.-L. and Botchan, M. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 569–584.
- Stringer, J.R. (1981) *J. Virol.* **38**, 671–679.
- Roth, D.B. and Wilson, J.H. (1986) *Mol. Cell. Biol.* **6**, 4295–4304.
- Pfeiffer, P. and Vielmetter, W. (1988) *Nucl. Acids Res.* **16**, 907–924.
- Nalbantoglu, J., Hartley, D., Phear, G., Tear, G. and Meuth, M. (1986) *EMBO J.* **5**, 1199–1204.
- Breimer, L.H., Nalbantoglu, J. and Meuth, M. (1986) *J. Mol. Biol.* **192**, 669–674.
- Nalbantoglu, J., Miles, C. and Meuth, M. (1988) *J. Mol. Biol.* **200**, 449–459.
- Roth, D.B., Chang, X.-B. and Wilson, J.H. (1989) *Mol. Cell. Biol.* **9**, 3049–3057.
- Rogers, J.H. (1985) *Int. Rev. Cytol.* **93**, 187–279.
- Sager, R., Gadi, I.K., Stephens, L. and Grabow, C.T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7015–7019.
- Colbère-Garapin, F., Ryhiner, M.-L., Stephany, I., Kourilsky, P. and Garapin, A.-C. (1986) *Gene* **50**, 279–288.
- Hoeijmakers, J.H.J., Odijk, H. and Westerveld, A. (1987) *Exp. Cell Res.* **169**, 111–119.