JOURNAL OF VIROLOGY, Feb. 1984, p. 445–451
0022-538X/84/020445-07$02.00/0

Vol. 49, No. 2

Chromosomal Sites of Integration of Simian Virus 40 DNA Sequences Mapped by In Situ Hybridization in Two Transformed Hybrid Cell Lines

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Received 23 May 1983/Accepted 12 October 1983

Simian virus 40 DNA sequences, integrated on human chromosome 7 in two transformed human-mouse hybrid somatic cell lines, were mapped to a specific chromosomal locus by in situ hybridization. To detect the integrated viral DNA by in situ hybridization, we increased the sensitivity of the technique by using as a probe ^{125}I-labeled simian virus 40 cRNA (2×10^9 to 3×10^9 dpm/μg) prepared by in vitro transcription of simian virus 40 DNA with high-specific-radioactivity [^{125}I]CTP. Although the viral nucleic acid was shown by blot hybridization in the two cell lines to be integrated in different restriction fragments, it was shown by in situ hybridization in the two cell lines to map to the same position, q31, on the long arm of human chromosome 7. The viral DNA integration sites were localized with a precision of ±1 silver grain diameter, equivalent to about 6.2×10^7 nucleotide pairs in the human genome. The procedures we describe may be adapted for localization of any gene on diploid chromosomes that can be cloned in a recombinant DNA vector.

The covalent integration of simian virus 40 (SV40) nucleic acid sequences into the genome of infected cells is the critical event responsible for persistence of viral transformation (33). The molecular mechanism by which SV40 DNA sequences integrate, however, remains unclear. One approach to understanding how integration occurs and how it affects the subsequent expression of both viral and host genetic information is to define the locus and arrangement of SV40 DNA sequences in different SV40-transformed cell lines.

Genetic analyses and nucleic acid hybridization studies have implied that SV40 DNA integration sites are apparently distributed in a random arrangement throughout the genome of transformed cells. Fusion of normal mouse cells with SV40-transformed human cells to produce stable hybrid somatic cell lines, in which human chromosomes have been preferentially eliminated, has permitted the assignment of integrated SV40 genes to specific human chromosomes. SV40 DNA and viral proteins have been shown to segregate, depending upon the hybrid cell line, with human chromosomes 5 (20), 7 (11, 36), 8 (22), 12 (10), and 17 (27), although the assignment to chromosome 8 has been disputed (10). Blot hybridization studies with different cell lines have revealed that the junction points between host and viral DNA map at different sites on the SV40 genome and that the number and arrangement of integrated viral genes may be different (3, 21). Direct sequence analysis of the integrated viral DNA and its host flanking sequences demonstrated that there is no homology between viral DNA sequences and host flanking DNA sequences (32). There is, however, some evidence for specificity of integration. Analysis by blot hybridization of the integrated viral DNA sequences in a series of rat cell lines that were independently transformed by SV40 under conditions in which the physiological state of the cells was similar revealed identical patterns in several transformants (28).

Our objective in this study was to map to a specific chromosomal locus by in situ hybridization the SV40 nucleic acid integration sites in two transformed human-mouse hybrid somatic cell lines. These hybrids have been shown to contain the entire mouse chromosome complement, but only one human chromosome, chromosome 7, to which the integrated viral genes were assigned (11, 36). Characterization of the SV40 DNA sequences in these cell lines by blot hybridization indicated that these two clones were derived from transformed human cells in which the viral DNA had become integrated at different sites on human chromosome 7 (5). We confirmed that these two cell lines were different clones by karyological analysis and by blot hybridization. To detect the SV40 nucleic acid integration sites by in situ hybridization, we increased the sensitivity of the technique. High-specific-radioactivity SV40 [^{125}I]cRNA (2×10^9 to 3×10^9 dpm/μg) was synthesized by in vitro transcription of SV40 DNA by using *Escherichia coli* RNA polymerase with high-specific-radioactivity [^{125}I]CTP. The [^{125}I]cRNA was then hybridized to the chromosomes of these cells in situ. After autoradiography, the loci of integration were determined by analysis of the positions of silver grains appearing over human chromosome 7. Our results show that the integrated viral nucleic acid sequences in these two cell lines map to position q31 on human chromosome 7.

MATERIALS AND METHODS

Materials. RNase A, polyadenylic acid [poly(A)], polyvinylpyrrolidone, ficoll, bovine serum albumin, and calf thymus DNA were purchased from Sigma Chemical Co. Ribonucleoside triphosphates (HPLC ULTRA) and aminopterin were purchased from ICN Pharmaceuticals. Thallic nitrate was purchased from Alfa Products, Na^{125}I and [α-^{32}P]dCTP were from New England Nuclear Corp., DNase I (DPFF) was from Worthington Diagnostics, restriction enzymes and nick translation reagents were from Bethesda Research Laboratories, Inc., formamide (Fluka purissima grade) was from Tridom Chemicals, and nitrocellulose was from Schleicher & Schuell Co. Standard saline phosphate-EDTA buffer (SSPE) contained 0.15 M NaCl–0.01 M NaH_2PO_4–0.001 M EDTA (pH 7.0). Standard saline citrate buffer (SSC) con-

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tained 0.15 M NaCl–0.15 M trisodium citrate (pH 7.0). BFP contained 0.2 mg each of bovine serum albumin, ficoll, and polyvinylpyrrolidone per ml (14). Form I SV40 DNA from strain C908 was purified from lytically infected A19 cells by the method of Hirt (19). *E. coli* RNA polymerase was purified by the method of Mangel (26) and was also kindly supplied by R. Burgess, University of Wisconsin, Madison.

Cells. The LN cell line (GM 2063) was purchased from the Human Genetic Mutant Cell Repository, Camden, N.J. The 53-87(3) cl.10 and 53-87(1) cl.21 cell lines were obtained from C. Croce, Wistar Institute, Philadelphia, Pa., and will be referred to as cl.10 and cl.21, respectively. The cl.10 and cl.21 hybrid cell lines were prepared by fusion of an uncloned population of SV40-transformed human fibroblasts, LN-SV, with normal mouse peritoneal macrophage cells from BALB/c and C57BL/6 mice, respectively (11, 36). The uninfected human fibroblast cell line, LN, was grown in Dulbecco modified Eagle medium with 10% fetal bovine serum, 100 U of penicillin per ml, 100 U of streptomycin per ml, and 0.25 μg of fungizone per ml. The cl.10 and cl.21 cell lines were grown in the same medium supplemented with 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine (24). The hybrid cell lines were SV40 T-antigen positive, and the LN cells were SV40 T-antigen negative, as detected by immunofluorescent staining (6) with anti-SV40 T-antigen immune serum supplied by Robert Carroll, New York University, New York.

Synthesis of [^{125}I]CTP. [^{125}I]CTP was prepared by modification of the methods of Dale et al. (12, 13). CTP was converted to 5-mercuro-CTP (Hg-CTP) and subsequently reacted with carrier-free Na^{125}I in the presence of a thallium catalyst to yield [^{125}I]CTP. The iodination reaction (20 μl) contained 0.1 M sodium acetate (pH 5.0), 10 μM Hg-CTP, 200 μM Tl $(NO_3)_3$, and 60 μM Na^{125}I. After incubation at 25°C for 30 min, the reaction was terminated by dilution with 0.5 ml of water. The reaction mixture was applied to a 0.1-ml DEAE-Sephadex A-25 column equilibrated in 0.05 M triethylammonium bicarbonate (pH 7.5). After washing extensively with the same buffer to remove unreacted Na^{125}I, the [^{125}I]CTP was eluted with 1.0 M triethylammonium bicarbonate (pH 7.5). The [^{125}I]CTP was desalted in vacuo aided by the addition of 75% ethanol, dissolved in water, and stored at 4°C. The specific radioactivity obtained was 1,600 to 2,100 Ci/mmol.

Synthesis of ^{125}I-labeled SV40 cRNA. The in vitro transcription reaction contained 50 mM Tris-hydrochloride (pH 7.9), 40 mM KCl, 10 mM $MgCl_2$, 0.25 mM dithiothreitol, 0.1 mM EDTA, 0.4 mM ATP, GTP, and UTP, 2 μM ^{125}I-CTP, 100 μg of SV40 DNA per ml, and 84 μg of *E. coli* RNA polymerase per ml. After incubation at 37°C for 90 min, the reaction mixture was adjusted to 100 mM sodium acetate (pH 5.0), 5 mM $MgSO_4$, and 10 mg of *E. coli* tRNA per ml, and the SV40 DNA was digested by incubation at 37°C for 10 min with 4 μg of DNase I per ml. The protein in the reaction mixture was extracted by adding an equal volume of phenol saturated with 0.01 M Tris-hydrochloride (pH 8.0)–0.1 M NaCl–0.05 M KI–5 mM EDTA. After centrifugation, the aqueous phase was applied to a 0.6- by 30-cm Sephadex G50 column equilibrated in the same buffer. [^{125}I]cRNA recovered in the void volume was precipitated with 2 volumes of ethanol, dried in vacuo, and dissolved in in situ hybridization buffer composed of 2× SSC, 50% (vol/vol) formamide, 1 mg of iodinated *E. coli* RNA per ml, 0.1 mg of poly(A) per ml, 0.1 mM KI, and 1× BFP.

Blot hybridization. High-molecular-weight cellular DNA was purified by modification of the method of Blin and Stafford (1) and digested by *Eco*RI, *Bam*HI, and *Bgl*II (5). The cleaved DNA was fractionated by electrophoresis for 22 h at 50 V in 0.7% agarose slab gels in Loening's buffer (25). The DNA fragments were transferred to nitrocellulose (34) and hybridized with ^{32}P-labeled, nick-translated SV40 DNA (7.1×10^7 dpm/μg) in 5× SSPE, 50% (vol/vol) formamide, 5× BFP, 100 μg of sonicated, denatured calf thymus DNA per ml, 50 μg of poly(A) per ml, 1 mM CTP, and 0.3% (wt/vol) sodium dodecyl sulfate at 35°C for 36 h. After hybridization, nitrocellulose filters were washed in two changes of 5× SSPE–50% (vol/vol) formamide–1× BFP–10 μg of poly(A) per ml–0.3% (wt/vol) sodium dodecyl sulfate at 35°C for 1 h each and then in several changes of 2× SSPE–0.2% (wt/vol) sodium dodecyl sulfate at 50°C. Hybridized bands were visualized by autoradiography.

In situ hybridization. The in situ hybridization procedure used was a modification of the procedures described by Steffensen (35) and Szabo et al. (37). Slides were pretreated (4), and metaphase chromosome spreads were prepared as described by Coté et al. (9). Slides were preincubated in 2× SSC at 70°C for 30 min (2), and endogenous RNA was removed by digestion with 0.1 mg of RNase A per ml in 2× SSC at 37°C for 1 h. Prehybridization buffer composed of 2× SSC, 50% (vol/vol) formamide, 10 mg of iodinated *E. coli* rRNA per ml, 1 mg of poly(A) per ml, 10 mM KI, and 10× BFP was overlaid on the cells, and the cells were covered with a glass cover slip. The slides were incubated in a buffer-saturated atmosphere within a sealed plastic bag at 35°C for at least 2 h, washed in 2× SSC, and dehydrated with ethanol. After acetylation of the slides with acetic anhydride (18), the DNA was denatured by heating the slides in 2× SSC–70% (vol/vol) formamide–10 mM KPO_4 (pH 7.0) at 70°C for 10 min, followed immediately by dehydration in ice cold 70% (vol/vol) ethanol in water. SV40 [^{125}I]cRNA in in situ hybridization buffer was applied over the cells, the cells were covered with a glass cover slip, and the slides were incubated as described above for 2 to 4 days. After hybridization, the cover slips were floated off the slides in 2× SSC–50% (vol/vol) formamide–0.1 M KI at 35°C. The slides were washed in 2× SSC–50% (vol/vol) formamide at 35°C for 1 h, rinsed in 2× SSC, and treated with RNase as above. After washing in a large volume of 2× SSC, the slides were dehydrated with ethanol, air dried, and autoradiographed with Kodak NTB emulsion (15). A more detailed description of the synthesis of [^{125}I]CTP and ^{125}I-labeled SV40 cRNA and the in situ hybridization procedure is in preparation.

RESULTS

Karyotypic analysis. Partial karyotypes of the cl.10 and cl.21 cells are shown in Fig. 1. Ten randomly selected metaphase plates were analyzed to determine the chromosome complement of each cell line. The mean number of mouse chromosomes present in the cl.10 cell line was 43 plus a single copy of human chromosome 7. The mean number of mouse chromosomes present in the cl.21 cell line was 77 plus two copies of human chromosome 7. Approximately half of the cells of both lines contained a distinctive metacentric marker chromosome that probably arose by fusion of two telocentric mouse chromosomes.

Analysis of the SV40 DNA sequences in high-molecular-weight cell DNA by blot hybridization. The cl.10 and cl.21 cell lines were previously shown to contain a single tandemly linked array of six SV40 genome equivalents per haploid chromosome complement (5). Blot hybridization patterns indicated that the SV40 nucleic acid sequences were inte-

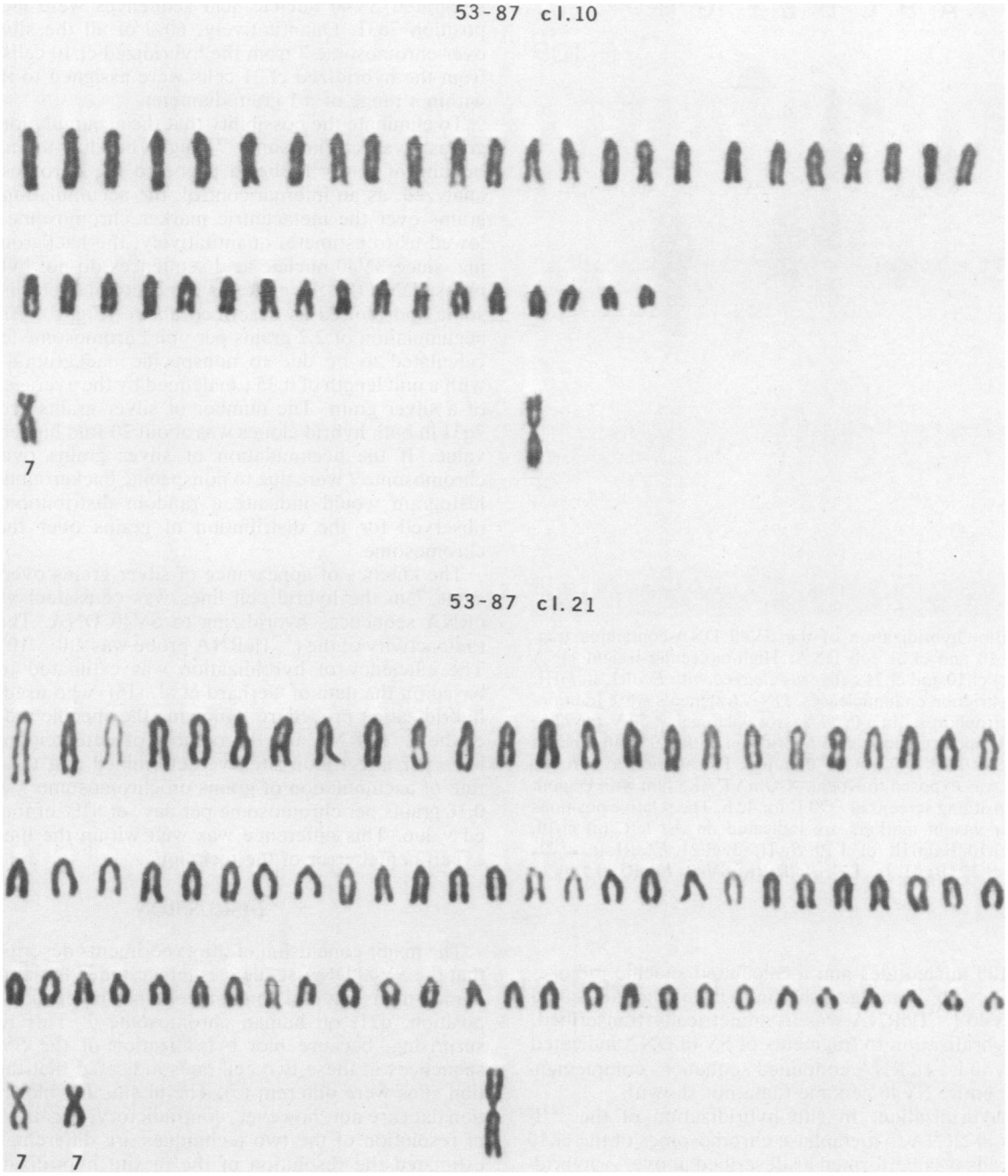

FIG. 1. Partial karyotype of the chromosomes in the hybrid cell lines: (a) 53-87(3) cl.10 and (b) 53-87(1) cl.21.

grated at different sites on human chromosome 7 in the two cell lines. We performed a similar analysis and confirmed that the viral integration sites in the cl.10 and cl.21 cells were different (Fig. 2). We also analyzed the high-molecular-weight DNA isolated from the nontransformed parent fibroblast cell line LN. No detectable hybridization was observed in the gel track containing the *Bgl*II-cleaved LN DNA, demonstrating that our SV40 DNA contained no human-specific sequences.

Synthesis of ^{125}I-labeled SV40 cRNA. A high-specific-radioactivity [^{125}I]cRNA probe was prepared by in vitro transcription of SV40 DNA by *E. coli* RNA polymerase with [^{125}I]CTP. [^{125}I]CTP was synthesized from Hg-CTP by a thallium-catalyzed electrophilic substitution of mercury with carrier-free Na^{125}I. Reaction yields, averaging 90% (2,000 Ci/mmol), were two- to sevenfold higher than previously reported (16), although there was apparently some Na^{125}I batch-dependent variability. Favorable reaction yields permitted efficient purification of high-specific-radioactivity [^{125}I]CTP by a single chromatographic step in contrast to the extensive isolation procedure of Hayashi et al. (17). Purified form I SV40 DNA was transcribed by *E. coli* RNA polymerase holoenzyme in reactions including 2 μM [^{125}I]CTP, a concentration of CTP at least 10-fold lower than the reported K_m for RNA chain elongation (30). A heterogenous mixture of [^{125}I]cRNA transcripts was obtained with an average

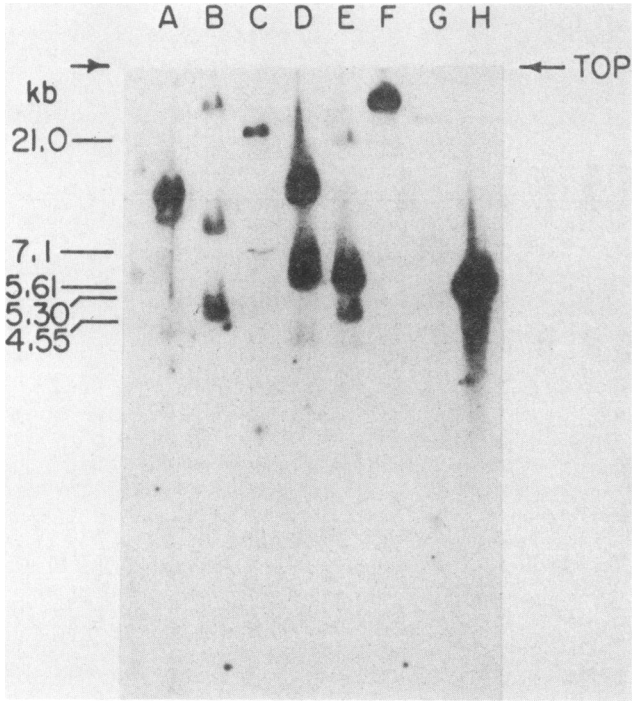

FIG. 2. Blot hybridization of the SV40 DNA-containing fragments in cl.10 and cl.21 cell DNA. High-molecular-weight DNA isolated from cl.10 and cl.21 cells was cleaved with *Eco*RI, *Bam*HI, and *Bgl*II restriction endonucleases. DNA fragments were fractionated by electrophoresis in a 0.7% agarose slab gel at 50 V for 22 h, transferred to nitrocellulose, and hybridized with 3.0×10^7 cpm of ^{32}P-labeled SV40 DNA (7.1×10^7 dpm/μg). The hybridized nitrocellulose sheet was exposed to Kodak X-OmAT AR5 film with Dupont Cronex intensifying screens at −70°C for 42 h. The relative positions of molecular weight markers are indicated on the left. (a) cl.10, *Eco*RI; (b) cl.10, *Bam*HI; (c) cl.10, *Bgl*II; (d) cl.21, *Eco*RI; (e) cl.21, *Bam*HI; (f) cl.21, *Bgl*II; (g) LN, *Bgl*II; (h) SV40, *Eco*RI, 0.1 ng of DNA.

length of 100 nucleotides and a calculated specific radioactivity of 2.5×10^9 dpm/μg. Solution hybridization indicated that the SV40 [^{125}I]cRNA was asymmetrically transcribed, and blot hybridization to fragments of SV40 DNA indicated that the SV40 [^{125}I]cRNA contained sequences complementary to the entire SV40 genome (data not shown).

In situ hybridization. In situ hybridization of the ^{125}I-labeled SV40 cRNA to metaphase chromosomes of the cl.10 and cl.21 cells was performed as described above. A hybridized chromosome spread from a cl.21 cell is shown in Fig. 3a. The human chromosome 7 from this spread is enlarged to illustrate the accumulation of silver grains over the locus of viral DNA integration (Fig. 3b). The cytogenetic localization of the SV40 DNA integration sites was determined in the following way. The relative position of individual silver grains was measured directly from photographic enlargements. Because the size of the silver grains varied significantly, the distance from the end of the chromosome to the center of each grain was used to assign the grain to a region of the chromosome. Grains that did not appear directly over the chromosome were projected to a position perpendicular to the horizontal axis of the chromosome. The number of grains appearing over human chromosome 7 was plotted as a function of their map position, resulting in the histograms shown in Fig. 4. In both the cl.10 and cl.21 cell lines the integrated SV40 nucleic acid sequences were localized to position 7q31. Quantitatively, 60% of all the silver grains over chromosome 7 from the hybridized cl.10 cells and 58% from the hybridized cl.21 cells were assigned to this locus, within a range of ±1 grain diameter.

To eliminate the possibility that the accumulation of silver grains over chromosome 7 might be due to nonspecific binding of the ^{125}I-labeled probe to the chromosome, we analyzed, as an internal control, the accumulation of silver grains over the metacentric marker chromosome. This allowed us to estimate, quantitatively, the background labeling, since SV40 nucleic acid sequences do not hybridize to mouse DNA (5). Silver grains were scored over this chromosome and plotted as described above (Fig. 5). An average accumulation of 2.2 grains per unit chromosome length was calculated to be due to nonspecific background labeling, with a unit length of 0.35 μm defined by the average diameter of a silver grain. The number of silver grains scored over 7q31 in both hybrid clones was about 20-fold higher than this value. If the accumulation of silver grains over human chromosome 7 were due to nonspecific background, then the histogram would indicate a random distribution, as was observed for the distribution of grains over the marker chromosome.

The kinetics of appearance of silver grains over chromosome 7 in the hybrid cell lines was consistent with SV40 cRNA sequences hybridizing to SV40 DNA. The specific radioactivity of the [^{125}I]cRNA probe was 2.0×10^9 dpm/μg. The efficiency of hybridization was estimated to be 2%, based on the data of Gerhard et al. (16), who used a similar hybridization procedure. Knowing the specific radioactivity of the [^{125}I]cRNA and the number of viral genome equivalents per integration site, we determined that the observed rate of accumulation of grains on chromosome 7 was about 0.16 grains per chromosome per day, or 81% of the calculated value. This difference was well within the limits of the experimental error of the technique.

DISCUSSION

The major conclusion of the experiments described here is that the SV40 DNA sequences integrated in two transformed human-mouse hybrid somatic cell lines mapped at the same position, q31, on human chromosome 7. This result was surprising, because blot hybridization of the SV40 DNA sequences in these two cell lines indicated that the integration sites were different (5). The in situ and blot hybridization data are not, however, contradictory, because the limits of resolution of the two techniques are different. We have estimated the resolution of the in situ hybridization technique at about ±1 silver grain diameter based on the experimental reproducibility observed in these studies. This range corresponds to approximately 1.2% of the human genome length, or about 6.2×10^7 nucleotide pairs. The limits of resolution of blot hybridization are on the order of a few hundred nucleotide pairs.

Control experiments were performed to demonstrate that the hybrid cell lines were different clones. Karyotypic analyses revealed that the two cell lines contained different chromosome complements. Comparison of blot hybridization patterns obtained by cleavage of cellular DNA with *Eco*RI, *Bam*HI, and *Bgl*II confirmed that the sites of integration of SV40 DNA on chromosome 7 were different in cl.10 and cl.21 cells. These results are in agreement with previously published data, from which it was concluded that these two hybrid cell lines were independently transformed and

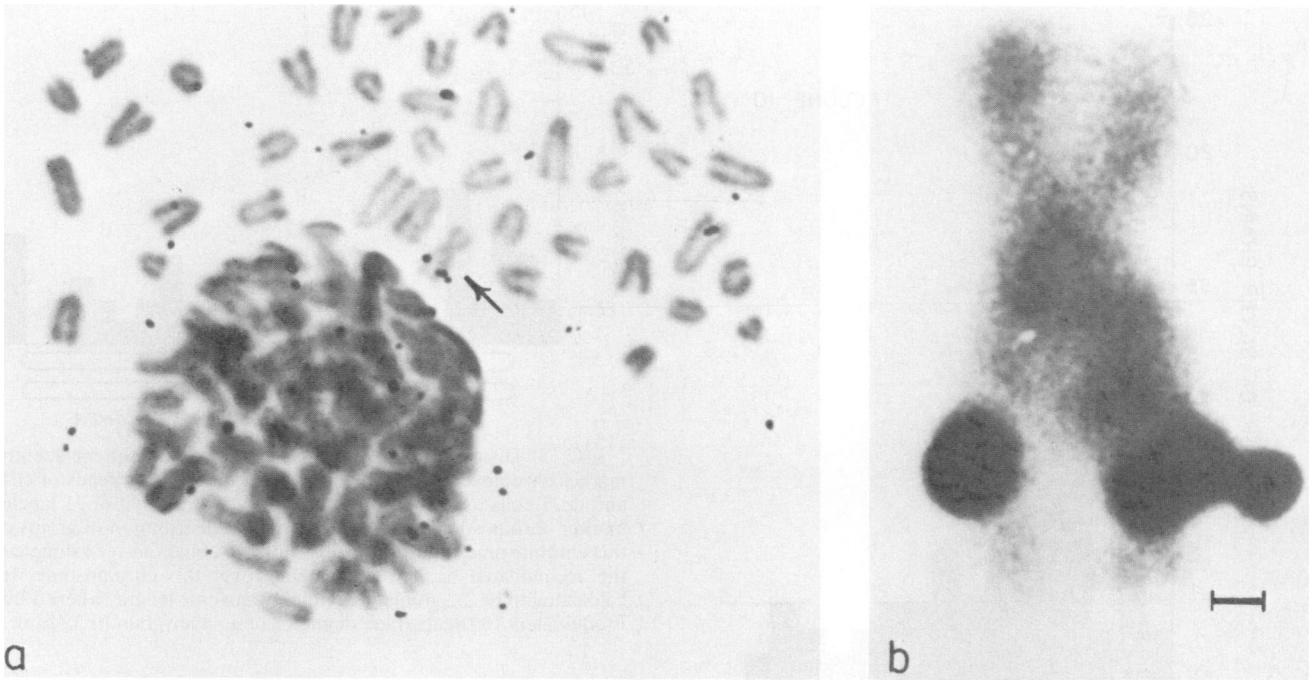

FIG. 3. In situ hybridization of SV40 [^{125}I]cRNA to metaphase chromosomes of a cl.21 cell. SV40 [^{125}I]cRNA (specific radioactivity, 2.0×10^9 dpm/μg) was hybridized to metaphase chromosomes at a concentration of 0.2 μg/ml for 72 h at 35°C. Hybridization was carried to a C_rt of 0.15 mol · s/liter. Slides were exposed for 16 days at 4°C. (a) Hybridized chromosome spread; arrow indicates human chromosome 7. (b) Human chromosome 7 enlarged to illustrate cytogenetic locus of SV40 DNA integration. Bar, 0.2 μm.

that the two copies of human chromosome 7 in the cl.21 cells were equivalent (5).

Additional control experiments demonstrated that the SV40 [^{125}I]cRNA probe hybridized specifically to viral DNA sequences on human chromosome 7. SV40 DNA isolated from lytically infected cells may contain integrated host DNA sequences (23). [^{125}I]cRNA obtained by in vitro transcription of the viral genome would therefore be comprised in part of human-specific nucleic acid sequences that could hybridize at equivalent positions on chromosome 7 in the two hybrid cell lines, resulting in identical cytogenetic localizations. This possibility was eliminated since no hybridization was detected between our SV40 DNA and the *Bgl*II-cleaved LN cell DNA. The observation of a significant autoradiographic signal over position 7q31, i.e., 20-fold higher than background, also implied that the probe was specifically hybridizing to a chromosomal DNA sequence at this site. The symmetrical distribution of grains around this site can be interpreted as arising from the random isotopic decay of [^{125}I]cRNA hybridized to this chromosomal locus. In addition, the kinetics of appearance of silver grains over this chromosomal site was found to be consistent with the kinetics of hybridization of SV40 cRNA sequences to SV40 DNA.

Current evidence implies that the integration of viral DNA into the genome of transformed cells results from an apparently nonspecific genetic recombination event. Such studies, however, are complicated by the possibility that the primary integration arrangement may be modified by secondary rearrangements that may occur during cell growth (7, 31). One way to resolve the question of the specificity of integration at the cytogenetic level is to map the primary locus of integration in transformed cells before any secondary rearrangements could occur. The data presented here indicate that such experiments are now possible with in situ hybridization.

The use of in situ hybridization for the localization of minimally reiterated gene sequences on mammalian diploid chromosomes has been, until recently, limited by technical problems. This limitation can primarily be attributed to the requirement for probes of extremely high specific radioactivities ($>10^9$ dpm/μg) to obtain a detectable autoradiographic signal at a specific genetic locus. For example, to map a single integrated copy of the SV40 genome in situ with a hybridization efficiency of 2% and an iodinated RNA probe of 3.5×10^9 dpm/μg would require a minimum autoradiographic exposure of 52 days to obtain an average of 3 silver grains at the integration site. Techniques for directly labeling purified RNA species with ^{125}I typically yield probes of 1×10^8 to 2×10^8 dpm/μg (8, 29). To obtain a probe of 3.5×10^9 dpm/μg would require that every cytidine residue in the nucleic acid be labeled. Our approach to solving this technical problem was to synthesize high-specific-radioactivity [^{125}I]CTP (2,000 Ci/mmol) and to use the [^{125}I]CTP in an in vitro transcription reaction to prepare ^{125}I-labeled cRNA with a specific radioactivity of 2×10^9 to 3×10^9 dpm/μg. If similarly prepared [^{125}I]UTP were used as well, the specific radioactivity could be doubled.

The use of extremely high-specific-radioactivity, nucleic acid probes for the localization by in situ hybridization of minimally reiterated genes of high base sequence complexity presented a new technical problem. Because of the high C_rt values required to achieve saturating hybridization in situ, greater than 10^6 cpm must be applied to each slide, and hybridization must be allowed to occur for several days. The large amount of radioactivity applied to each slide can result in prohibitively high levels of nonspecific background labeling if standard in situ hybridization procedures are used. To

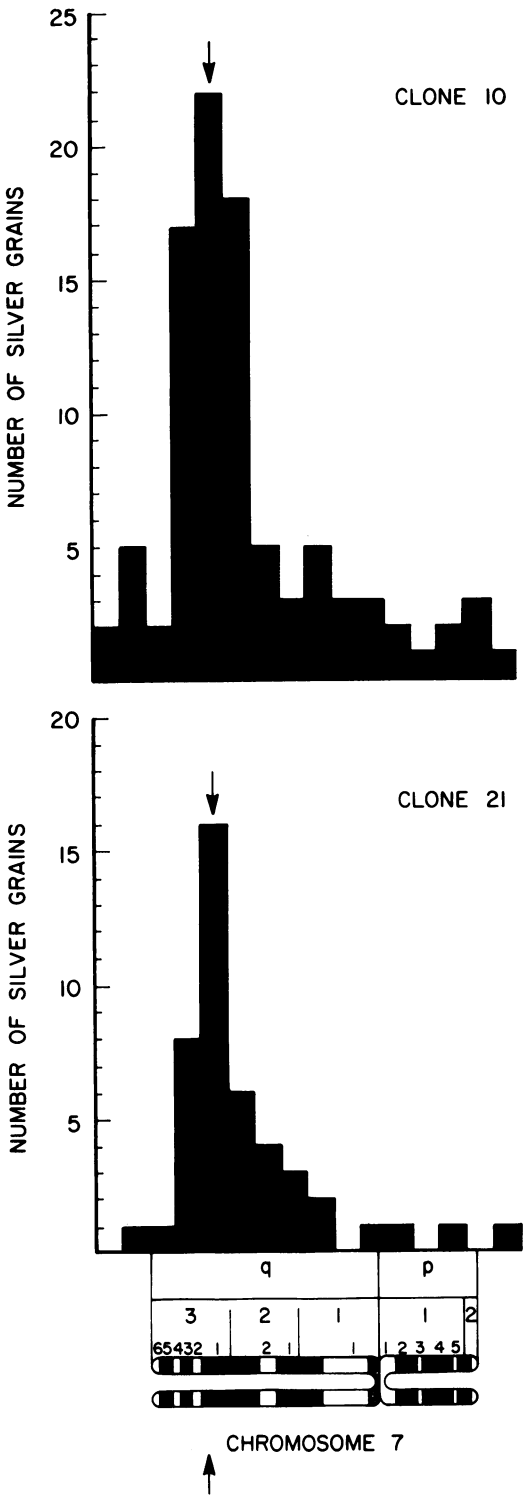

FIG. 4. Distribution of silver grains scored over human chromosome 7 as a function of map position in hybridized metaphase chromosome spreads of cl.10 and cl.21 cells. (a) Localization of integrated SV40 DNA sequences to position 7q31 in cl.10 cells. A total of 32 labeled human chromosomes were analyzed, resulting in assignment of 60% of all grains scored to this locus. A range of ±1 grain diameter is assumed. (b) Localization of integrated SV40 DNA sequences to position 7q31 in cl.21 cells. On the 48 labeled human chromosomes analyzed, 58% of all grains mapped to a site indistinguishable from that observed in the cl.10 cells.

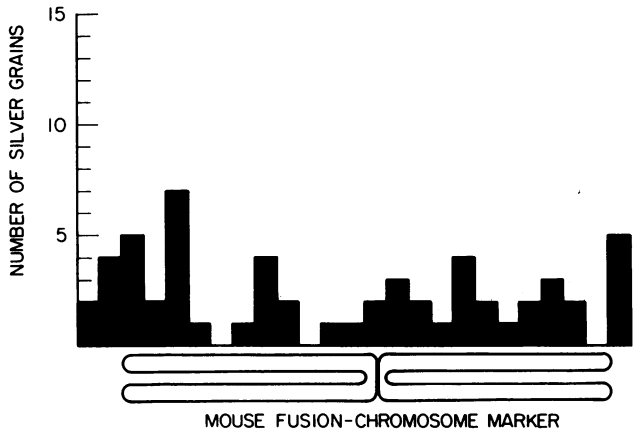

FIG. 5. Distribution of silver grains scored over the metacentric marker chromosome from metaphase chromosome spreads of cl.10 and cl.21 cells as a function of map position. A total of 31 labeled marker chromosomes were analyzed. The distribution of grains on this chromosome was apparently random. A quantitative estimate of the accumulated background labeling over this chromosome was calculated to be 2.2 grains per unit chromosome length, where 1 unit is equivalent to the average diameter of a silver grain (0.35 μm).

minimize the background, we modified the standard hybridization procedure by including pretreatment of the slides (4), acetylation of the cytological preparations (18), and the addition of BFP (14) and iodinated nucleic acid carriers to the hybridization buffers. Although we have used this procedure to study viral transformation, the modified techniques we have developed may easily be adapted to map on diploid chromosomes any cloned nucleic acid sequence that can be propagated in a recombinant DNA vector.

ACKNOWLEDGMENTS

This paper is dedicated to the memory of B. D. Coté.

This research was supported by a grant from the Earl M. Bane Estate (to W.F.M.) and by Public Health Service grants GM 25083 (to O.C.U.), GM 21123 (to D.M.S.), and CA 25633 (to W.F.M.) from the National Institutes of Health.

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