Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8338–8342, November 1986
Medical Sciences

Hepatitis B virus integration site in hepatocellular carcinoma at chromosome 17;18 translocation

(hepatocarcinogenesis/chromosomal aberration)

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Communicated by Frank Lilly, July 1, 1986

ABSTRACT **Integrated hepatitis B virus (HBV) DNA is almost invariably found in hepatocellular carcinomas (HCC) which develop in HBV carriers. Integrated HBV DNAs from two single-integration HCCs (C3 and C4) have been cloned, and the cellular integration sites have been analyzed. Integrated HBV DNA of C3 is present in chromosome 6 and contains a nearly complete linear HBV genome. The HBV DNA integration in tumor C3 was not associated with major rearrangements of cellular DNA. In contrast, the integrated HBV DNA in C4 contains a large inverted repeat of HBV DNA, in which each repeat consists of a linear HBV DNA segment similar to that present in C3. The C4 integration was also accompanied by a cellular DNA translocation at the HBV integration site. The translocation occurred between chromosomes 17 and 18, along with a deletion of at least 1.3 kilobases of chromosome 18 DNA at the translocation site. Our data support a model in which postintegration rearrangement of integrated HBV and cellular DNA results in the generation of chromosomal aberrations. These chromosomal aberrations may function in a multistage mechanism leading to fully malignant HCC.**

Epidemiologic studies have revealed a strong correlation between the occurrence of hepatocellular carcinoma (HCC) and endemic hepatitis B virus (HBV) infection in human populations worldwide. Specifically, chronic HBV surface antigen (HBsAg)-positive carriers have up to 200-fold greater risk of developing HCC than uninfected individuals from the same area (1). The discovery that host DNA from most primary HCCs contained integrated HBV DNA stimulated interest in the possible role of viral DNA integration in hepatocarcinogenesis (2–7). Both HBV and the closely related woodchuck hepatitis virus (WHV) can integrate into cellular DNA during chronic infection, preceding HCC (2, 5, 8). This is consistent with their potential action as tumor initiators (9). Hepadnaviruses so far have not been shown to contain a viral oncogene per se, nor has a common cellular integration site been identified in HCCs (7, 10–12). In addition, some human and woodchuck HCCs from carriers do not contain any integrated or free viral DNA (6, 7, 13). These findings raise the possibility that viral antigens are not required for the maintenance of HCC and may function only as tumor initiators. Additional studies are necessary to identify a unifying molecular mechanism to explain the strong epidemiological data linking chronic infection with HCC.

Factors that stimulate the incidence of chromosome aberrations are associated with increased risk of neoplasia (9, 14). In the case of chemical hepatocarcinogenesis in the mouse and rat, agents that damage DNA stimulate the occurrence of HCC (15–18). The ability of oncogenic viruses to damage DNA through integration and by increasing the mutation rate of cellular genes is probably an important factor in virus-mediated multistage carcinogenesis (19). To investigate the possible role of HBV integrations in a general mechanism involving host DNA rearrangements, we have studied the structure of cellular DNA sequences at HBV integration sites. A previous study (20) reported a large deletion of cellular DNA at chromosome position 11p13 in association with an HBV integration. In this report we describe an HBV-induced translocation between chromosomes 17 and 18. We believe it is the first translocation to be directly linked to any viral DNA integration. The ability of HBV integrations to generate chromosome defects may be part of a multistep mechanism in the development of fully malignant HCC.

METHODS

Primary HCCs were obtained from two HBV carriers at autopsy. The first tumor, C3, was obtained from a 62-year-old Japanese man and the second, C4, was obtained from a 37-year-old Japanese man. Both were HBsAg-positive, and both tumors had a trabecular phenotype.

DNA was extracted from the tumors, and restriction endonuclease fragments containing integrated HBV DNA were identified by Southern blot analysis as previously reported (3). Tumors C3 and C4 each contained only a single site of HBV integration. *Hin*dIII-digested tumor DNAs were size-fractionated and specific size fractions were ligated into *Hin*dIII arms of λ phage Charon 30 and packaged *in vitro* (3, 21). The recombinant phage library was screened with purified ^{32}P-labeled HBV probe as described (3), and positive clones were plaque-purified and designated clones C3 and C4 accordingly.

Restriction endonuclease maps of the clones in this report were determined by Southern blot analysis. Restriction fragments from clones C3 and C4 corresponding to unique cellular flanking sequences were isolated and used as probes in genomic-mapping studies. The restriction fragments used as probes are denoted by brackets below the restriction maps of integrated HBV DNA in Figs. 2 and 3.

RESULTS

Structure of Integrated HBV DNA in Clones C3 and C4. Tumors C3 and C4 each contained a single site of HBV DNA integration according to Southern blot analysis with *Hin*dIII, which does not cleave the HBV genome. Episomal forms of HBV DNA were not observed in the tumors. Each integration was cloned into λ phage Charon 30 at the *Hin*dIII site. Southern blot analysis of genomic DNA, using *Hin*dIII and other enzymes, indicated that the integrated-HBV clones

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Abbreviations: HBV, hepatitis B virus; HBsAg, HBV surface antigen; HBcAg, HBV core antigen; WHV, woodchuck hepatitis virus; HCC, hepatocellular carcinoma; bp, base pair(s); kb, kilobase(s).

were identical in structure to the integrated HBV DNA present in the original tumors. Restriction endonuclease maps of clones C3 and C4 were determined by Southern blotting with ^{32}P-labeled HBV probe (Fig. 1). The structural organization of integrated HBV DNA was determined according to the known restriction map of the HBV genome. The restriction map of the integrated sequences was similar to that of HBV clones of the Adr subtype, which is common in Japan (22). Electron microscopic heteroduplex analysis of the purified inserts with cloned HBV DNA confirmed the restriction endonuclease maps (data not shown).

Both viral–cellular junctions of the C3 integration were in the cohesive overlap region of the HBV genome. The cohesive overlap is defined by a pair of 11-bp direct repeats, designated DRI and DRII, which are located at nucleotides 1824 and 1590, respectively, on the circular HBV genome map (Fig. 1*c*). The integrated DNA in clone C3 is a nearly unit-length HBV genome. Clone C4 contains 6.0 kb of integrated HBV DNA comprised of two ≈3.0-kb segments in inverted orientation (Fig. 1*b*). Each 3.0-kb repeat is colinear with the HBV genome, and the viral–cellular DNA junctions are also in the cohesive overlap region. The inversion occurred at the 5′ end of the core antigen (HBcAg) gene. Each 3.0-kb segment of HBV in C4 closely resembles the linear HBV DNA present in clone C3. Since all four of the viral–cellular junctions in clones C3 and C4 are located in the cohesive overlap of HBV, a specific integration mechanism is implicated (see *Discussion*).

C3 HBV Integration Site. Restriction endonuclease fragments containing unique cellular DNA were isolated from the left and right flanking sequences of clones C3 and C4 and these were used to probe Southern blots of leukocyte and tumor DNAs. Southern blots of leukocyte DNA hybridized with the left and right flanking probes from clone C3 showed that both probes hybridized to common-size *Hin*dIII, *Eco*RI, and *Bam*HI genomic DNA fragments (Fig. 2). The 1.6-kb *Hin*dIII fragment that hybridized to both probes was the length of the cellular sequences in clone C3, as determined by subtracting the length of integrated HBV DNA (≈3.1 kb) from the total length of the clone (4.7 kb) (Fig. 2). This indicated that rearrangement of cellular sequences had not occurred during HBV integration. This conclusion was also supported by *Bgl* II digestion data. The left-hand probe hybridized to a 1.1-kb *Hin*dIII–*Bgl* II fragment in leukocyte DNA that was the expected size for a *Hin*dIII–*Bgl* II fragment spanning the HBV integration site.

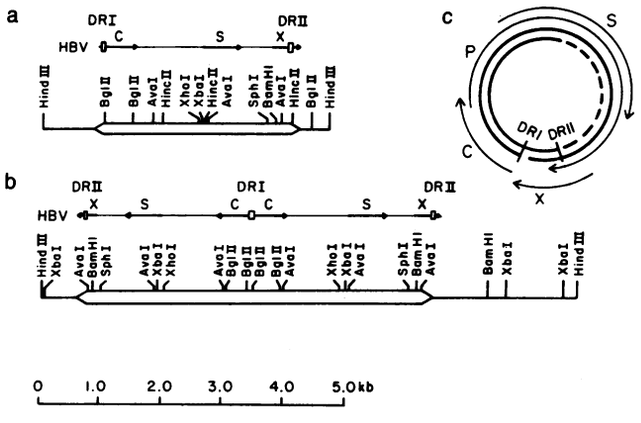

FIG. 1. (*a* and *b*) Restriction endonuclease maps and genetic organization of integrated HBV DNA sequences in clones C3 (*a*) and C4 (*b*). The HBV genetic maps above each clone denote the location of HBV genes in that clone. (*c*) Circular HBV genome map. Broken line represents single-stranded region in HBV virion DNA. HBV genome markers include DRI and DRII, which are 11-base-pair (bp) direct repeats and the termini of the cohesive overlap region; C, HBcAg gene; S, HBsAg gene; X, X gene; P, polymerase gene. In *a* and *b*, open bars represent integrated HBV DNA, whereas flanking cellular DNA is represented by lines. kb, Kilobases.

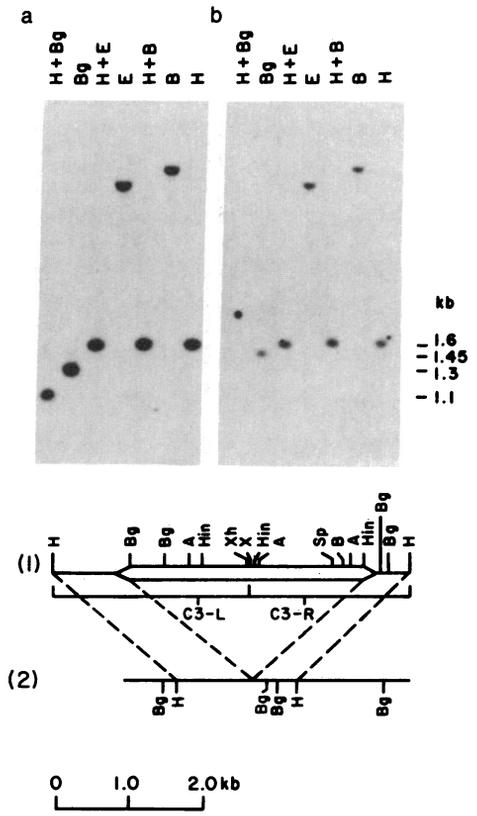

FIG. 2. Analysis of normal cellular DNA at the C3 HBV integration site. (*Upper*) Southern blots of leukocyte DNA hybridized with C3 left probe (*a*) or C3 right probe (*b*). (*Lower*) Restriction fragments from clone C3 used for probes were the left and right *Hin*dIII–*Xba* I fragments (C3-L and C3-R), denoted by brackets below restriction endonuclease map of clone C3 (line 1). Integrated HBV DNA and cellular DNA are represented as in Fig. 1. Line 2 shows restriction endonuclease map of the normal cellular DNA integration site. Restriction endonuclease abbreviations: H, *Hin*dIII; Bg, *Bgl* II; A, *Ava* I; Hin, *Hin*cII; Xh, *Xho* I; X, *Xba* I; Sp, *Sph* I; B, *Bam*HI; E, *Eco*RI.

C4 HBV Integration Site. Since *Bgl* II and *Hin*dIII did not cut cellular sequences within clone C4, we expected that the probes from the right- and left-hand cellular DNA should hybridize to common-size *Bgl* II and *Hin*dIII fragments in normal DNA if no rearrangement of cellular sequences had occurred at the HBV integration site. However, hybridization of the C4 left and right flanking probes with leukocyte DNA revealed hybridization to different-size restriction endonuclease fragments, leading to the conclusion that the sequences were not contiguous in normal DNA (Fig. 3 *Upper*). The restriction map of clone C4, in combination with Southern blot data of leukocyte DNA, allowed us to construct restriction maps of the normal cellular loci homologous to the right and left flanking probes (Fig. 3 *Lower*). Hybridization of the C4 flanking probes to Southern blots of C4 tumor DNA confirmed the restriction map of the HBV integration site that had been deduced from leukocyte DNA data. Therefore, we concluded that a rearrangement of cellular sequences, either a deletion or a chromosomal translocation, had occurred at the HBV integration site.

Chromosomal Localization of C3 and C4 Integrations. The chromosomal location of the C3 and C4 flanking probes was determined by hybridizing them simultaneously to Southern blots of *Bgl* II-digested human–mouse somatic cell hybrid

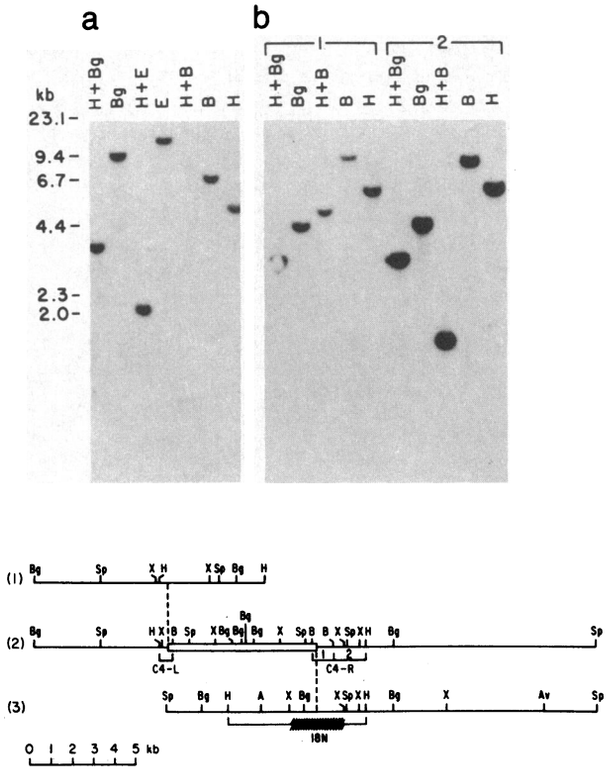

FIG. 3. Analysis of normal cellular DNA at the C4 HBV integration site. (*Upper*) Southern blots of leukocyte DNA hybridized with C4 left probe C4-L (*a*) or with right-hand cellular DNA probes C4-R1 (*b*, lanes 1) and C4-R2 (*b*, lanes 2). Markers at left indicate positions of *Hin*dIII fragments of bacteriophage λ DNA run in parallel. (*Lower*) Line 1 is a restriction map of the normal chromosome 17 DNA across the HBV integration site. Line 2 is a restriction map of integrated HBV DNA and cellular DNA from tumor C4. Brackets below line denote probes used to map cellular loci. Probe C4-L comprised the left-hand *Hin*dIII–*Bam*HI fragment. C4-R1 was the right-hand *Bam*HI HBV–cellular DNA junction fragment. C4-R2 was the right-hand *Hin*dIII–*Bam*HI fragment, containing only cellular DNA sequences. Line 3 is a restriction map of normal chromosome 18 DNA across the HBV integration site. Bracket below line 3 denotes the *Hin*dIII fragment comprising clone 18N, which is the normal right-hand locus spanning the HBV integration site. The hash-marked region is the 2.5-kb *Xba* I fragment used as a probe for chromosome 18 sequences in tumor C4 (Fig. 4). Restriction endonuclease abbreviations are as in Fig. 1.

DNAs. The somatic cell hybrid DNAs were from cell lines that contained defined complements of human chromosomes. The probes for C4 left, C4 right, and C3 left hybridized to *Bgl* II fragments of 9.7, 4.2, and 1.3 kb, respectively. Hybridization analysis of a panel of somatic cell hybrids with the C3 left probe enabled us to tentatively localize it to chromosome 6, with a 10% discordancy rate. The results showed that the C4 left and C4 right probes did not cosegregate in several somatic cell hybrids and thus were present on different chromosomes. Hybridization analysis of the somatic cell hybrid panel enabled us to locate the left and right C4 probes on chromosomes 17 and 18, respectively (Table 1). Therefore, we concluded that a chromosomal translocation had occurred at the HBV integration site in tumor C4.

Deletion at C4 Translocation Site. The mechanism by which the translocation occurred was studied by cloning the normal cellular sequence that spanned the translocation breakpoint in chromosome 18. A library of Charon 30 recombinant phage containing *Hin*dIII-digested normal DNA fragments was screened with the *Hin*dIII–*Bam*HI right-hand C4 probe. A clone (designated 18N) containing a 6.5-kb *Hin*dIII insert was isolated that contained the normal DNA across the chromosome 18 breakpoint (Fig. 3 *Lower*, line 3 bracket). The only DNA segment of this clone that did not contain highly repeated DNA sequences was the 2.5-kb *Xba* I fragment that spanned the breakpoint on chromosome 18 (Fig. 3 *Lower*, line 3 hatch marks). This DNA fragment was isolated and used as a hybridization probe to test whether reciprocal recombination had occurred during the translocation event. We predicted that, if reciprocal recombination had occurred, we would observe three DNA fragments hybridizing to the 18N probe when it was hybridized to C4 tumor DNA. These fragments would correspond to (*i*) the right-hand normal sequence, (*ii*) the C4 right-hand HBV junction fragment (chromosome 18), and (*iii*) the predicted chromosome 17–18 reciprocal recombination fusion fragment comprising sequences to the left of the chromosome 18 breakpoint and to the right of the chromosome 17 breakpoint.

Hybridization of the 18N probe to C4 tumor and normal cellular DNAs revealed the presence of only two fragments, which corresponded to (*i*) the right-hand HBV junction fragment and (*ii*) the normal allele (Fig. 4). We could not detect the third predicted reciprocal recombination fusion fragment in any of the digests we tested. This was particularly evident for the *Sph* I digest of C4 tumor DNA, in which the normal 8.5-kb fragment and the 1.9-kb HBV junction fragment were identified but the proposed recombination fragment of 9.4 kb was not observed (Fig. 4). We therefore concluded that a deletion of ≥1.3 kb of cellular DNA (through the *Xba* I site to the left of the chromosome 18 breakpoint) had occurred before or during the initial HBV integration or during the translocation event. The deleted sequences must have been lost from the tumor cell, otherwise a third fragment would have been observed in our experiment.

DISCUSSION

The HBV viral–cellular and viral–viral junctions in clones C3 and C4 have been located in the cohesive overlap of the HBV genome by restriction endonuclease and heteroduplex analysis. In the case of integration C3, an almost complete linear HBV genome is present, with one viral–cellular DNA junction near the 5′ end of the HBcAg gene and the other near the 3′ end of the X gene of HBV. This structure may have resulted from the integration of a viral replication intermediate that had completed minus-strand DNA synthesis. Minus-strand DNA replication intermediates contain a protein primer at their 5′ end (36). Cleavage of the protein primer plus a small segment of the virion DNA from the 5′ end of a DNA minus strand during integration would produce at least one viral–cellular junction in the cohesive overlap region of HBV. If a complete minus strand served as the source for integrated DNA, the other viral–cellular junction should be in the DRI sequence of HBV. DNA sequencing has shown that this is the case (data not shown). An integration mechanism involving replication intermediates has been proposed (26) and predicts the absence of terminal duplication of sequences in the cohesive overlap region in any single linear HBV integration, as was observed for integration C3.

Integrated HBV DNA in clone C4 resembles an inverted repeat of HBV DNA sequences present in clone C3. This leads to the hypothesis that the inverted duplication was generated by recombination between two initially separate integrated DNAs on separate chromosomes. We investigated the mechanism by which the C4 translocation was generated by cloning the normal DNA across the chromosome 18 breakpoint. If the translocation involved a reciprocal exchange of chromosome 17 and chromosome 18 sequences, a second 17;18 translocation chromosome should have been present in tumor C4, which would not contain any integrated HBV DNA (only a single integrated HBV DNA was present in tumor C4). To search for the second 17;18 fusion product,

Table 1. Distribution of cellular DNA sequences (C3-R, C4-L, and C4-R2) in human–mouse cell hybrids

Hybrid	Flanking-sequence probes			Human chromosomes																						Chromosomal alteration(s)
	C4-L	C4-R2	C3-R	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
AIR-13	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	t	t(5;X)
DUA-3BSAGA	+	−	−	−	+	−	−	−	−	+	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	
DUA-5BSAGA	+	+	+	−	−	+	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	+	−	−	
DUM-13	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	−	+	t	+	+	+	+	+	+	+	t	rcpt(X;15)
EXR-5CSAz	+	+	+	+	+	+	+	+	+	+	+	+	+	t	+	+	+	+	−	+	+	+	+	+	+	+	t(X;11)
ICL-15CSBF	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	
JSR-14	+	−	?	−	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	+	
JSR-17S	+	+	−	+	+	+	−	+	−	t	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	t(7;9)
JWR-22H	+	+	+	t	t	−	+	−	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	−	−	t(2;1)
JWR-26C	+	+	+	t	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	t(1;2)
NSL-9	+	−	−	−	−	−	−	+	−	−	+	t	+	−	+	+	+	+	+	+	−	−	+	+	+	−	t(17;9)
REW-7	+	+	?	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
REW-80	+	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	+	+	
REW-10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
REW-11	−	−	−	−	−	−	+	−	−	+	−	−	−	+	+	+	−	−	+	−	−	−	+	+	+	+	
REW-15	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
REX-11BSAgB	−	+	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−	−	−	
REX-11BSHF	−	+	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	t	t	t(22;X)
SIR-8	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	
TSL-1	+	+	−	−	−	+	+	−	−	−	−	+	+	+	−	+	+	−	+	+	+	−	+	+	−	−	
TSL-2	+	+	+	−	+	t	−	+	+	−	−	−	+	−	+	−	−	−	−	t	+	−	+	+	−	+	rcpt(3;17)
VTL-6	+	−	+	−	+	−	−	−	+	+	+	−	+	+	−	−	−	+	−	+	−	+	+	+	−	−	
VTL-7	+	−	−	−	−	−	−	−	−	t	−	−	−	+	−	−	+	−	−	+	−	+	+	+	+	−	7q−
WIL-1	+	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	+	−	−	+	+	−	−	+	−	+	
WIL-1	+	?	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	
WIL-7	+	+	+	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	
WIL-8X	+	+	−	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	+	
WIL-13	+	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	
WIL-14	+	−	+	+	−	+	−	+	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	
WIL-15	+	+	+	−	+	+	+	−	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	−	+	
XER-7	−	+	+	+	+	+	+	+	+	+	+	+	+	t	+	+	+	+	−	−	+	+	−	−	−	+	t(11;X)
XER-11	+	+	+	+	−	+	+	−	+	+	+	−	+	t	+	+	−	+	+	+	+	+	+	+	+	t	rcpt(11;X)
XTR-22	−	+	+	−	+	t	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	+	t(X;3)
C4-L discordance																											
No. of concordant hybrids				15	19	20	17	21	18	19	20	11	21	18	22	19	24	18	14	32	21	14	21	27	15	17	
No. of discordant hybrids				16	13	11	16	12	15	12	13	21	12	12	12	11	9	14	19	0	12	19	12	6	17	12	
% discordant				52	41	35	48	36	45	39	39	66	36	40	33	42	27	44	58	0	36	58	36	18	53	41	
C4-R discordance																											
No. of concordant hybrids				18	20	26	20	22	21	17	16	15	26	20	20	18	23	20	15	20	32	17	16	21	13	18	
No. of discordant hybrids				12	11	4	12	10	11	13	16	16	6	9	12	14	9	1	17	11	0	15	16	11	18	10	
% discordant				40	35	13	38	31	34	43	50	52	19	31	37	44	28	35	53	35	0	47	50	34	58	36	
C3-R discordance																											
No. of concordant hybrids				20	23	19	19	21	28	20	17	14	22	18	20	17	15	23	14	18	20	21	17	17	12	19	
No. of discordant hybrids				9	7	10	12	10	3	9	14	16	9	10	11	14	16	7	17	12	11	10	14	14	18	8	
% discordant				31	23	34	39	32	10	31	45	53	29	36	35	45	52	23	55	40	35	32	45	45	60	30	

The human–mouse hybrid cell panel was derived from independent sets of hybrids constructed with 4 mouse cell lines and 12 human fibroblast lines (23, 24). Chromosomes of hybrid cells were karyotyped and banded by Giemsa/trypsin staining (25); "t" indicates that a translocated piece of a chromosome was present, but not the intact chromosome. Enzyme markers assigned to each chromosome except Y have been tested on each cell hybrid (24) to confirm the chromosome analysis. Chromosomes, enzymes, and the probes were tested on the same cell passage for each hybrid. Most hybrids were derived from karyotypically normal human parental cells; the others were derived from parental cells with well-defined translocation chromosomes for regional chromosome mapping. All three probes were hybridized simultaneously to Southern blots of *Bgl* II-digested DNA from each cell hybrid; scoring indicates the presence (+) or absence (−) of human bands on the blots. Discordant hybrids either retained the sequences but not a specific chromosome or vice versa.

we used normal DNA across the chromosome 18 breakpoint as a hybridization probe of C4 tumor DNA. We were not able to detect the extra restriction fragments homologous to the second reciprocal recombination chromosome that were predicted. This suggested that a deletion of host DNA sequences had also occurred in addition to the translocation. The deletion must have encompassed at least 1.3 kb of DNA immediately to the left of the breakpoint on chromosome 18, otherwise our probe would have detected an additional restriction fragment.

The deletion of host DNA sequences, in association with a chromosomal translocation, has been reported for a translocation of the c-*myc* gene to the immunoglobulin locus in a murine plasmacytoma (27). Also, integrated HBV and WHV DNAs from human and woodchuck HCCs most often have undergone multiple rearrangements of viral sequences, including deletions and direct or inverted duplications (3, 10, 11, 21, 28, 29). Many of the viral–viral and viral–cellular junctions in these integrations are not located in the cohesive overlap region. The results in this report and sequencing data

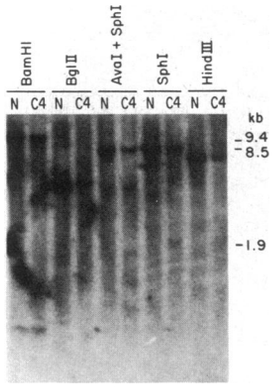

FIG. 4. Analysis of translocation fragments in tumor C4 DNA. Southern blot of normal pancreas (N) and C4 tumor (C4) DNAs digested with various enzymes and hybridized with the 18N probe spanning the chromosome 18 breakpoint. (Probe description in Fig. 3 *Lower*, line 3.) Numbers at right are the sizes of bands for *Sph* I-digested C4 tumor DNA predicted by the reciprocal recombination model. The predicted 9.4-kb reciprocal recombination fusion fragment was not observed.

across the HBV inversion in clone C4 (data not shown) show that the translocation was not generated by homologous recombination between separate integrated HBV DNAs and that another mechanism, possibly involving a breakage and reunion event, probably generated the translocation.

The absence of integrated HBV DNA in some tumors shows that HBV gene products are most likely not required for the maintenance of HCC. Therefore, if the virus functions as a tumor initiator, it is most probably one that may be lost from the cell after initiation has occurred. This would be similar to mechanisms proposed for several persistent DNA viruses that can cause cellular DNA rearrangements by a "hit-and-run" mechanism (30, 31). In one report (32), three cell lines transformed with adenovirus type 12 showed loss of all integrated viral DNA. These cells maintained their oncogenic phenotype, but they reverted from an epithelial morphology to a fibroblastic morphology. A related hit-and-run mechanism is probably responsible for herpes simplex virus transformation. All human herpesviruses induce chromosomal aberrations (19, 33) and recent work has shown that the transforming sequences of herpes simplex virus types 1 and 2 and of cytomegalovirus contain DNA sequences with structural characteristics similar to insertion-sequence elements (34). Restriction enzymes that cut within these elements destroy the transformation activity, possibly by preventing their action as insertion sequences.

In general, once HCC cell lines are established, the HBV integration pattern is quite stable. However, amplification and transposition of integrated HBV plus flanking cellular sequences has been reported in the PLC/PRF/5 hepatoma cell line (28, 35). Whether this transposition occurred in the primary tumor or during establishment of the cell line is unknown. Mizusawa *et al.* (29) have also reported the inverted duplication of HBV and cellular sequences cloned from a human hepatoma cell line. The translocation in this report and an earlier report (20) of deletion of cellular DNA associated with HBV integration show that HBV integrations may serve as focal points for the generation of chromosomal aberrations present in primary HCC. The specific function of these aberrations is not understood. However, the well-documented role of chromosomal aberrations in carcinogenesis, in general, suggests that they play a role in a multistep mechanism leading to fully malignant HCC.

We thank Drs. T. Kitagawa, H. Sugano, and D. Shafritz for encouragement throughout this work. We thank R. Eddy, L. Haley, M. Byers, and W. Henry for technical assistance. The work reported in this paper was undertaken during the tenure of a Yamagiwa–Yoshida Memorial International Cancer Study Grant awarded by the International Union Against Cancer. This work was also supported by a grant-in-aid for cancer research and a grant-in-aid for special project research (Cancer–Bioscience) from the Ministry of Education, Science, and Culture of Japan; by Public Health Service Grants CA37232-02 (to C.E.R.) and GM20454 and HDD5196 (to T.B.S.); and by Grants JFRA-100 and CD-62 from the American Cancer Society.

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