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Genetics

Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas

(viral DNA integration/c-*myc*/genital cancer)

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ABSTRACT **The chromosomal locations of cellular sequences flanking integrated papillomavirus DNA in four cervical carcinoma cell lines and a primary cervical carcinoma have been determined. The two human papillomavirus (HPV) 16 flanking sequences derived from the tumor were localized to chromosome regions 20pter→20q13 and 3p25→3qter, regions that also contain the protooncogenes c-*src*-1 and c-*raf*-1, respectively. The HPV 16 integration site in the SiHa cervical carcinoma-derived cell line is in chromosome region 13q14→13q32. The HPV 18 integration site in SW756 cervical carcinoma cells is in chromosome 12 but is not closely linked to the Ki-*ras2* gene. Finally, in two cervical carcinoma cell lines, HeLa and C4-I, HPV 18 DNA is integrated in chromosome 8, 5′ of the c-*myc* gene. The HeLa HPV 18 integration site is within 40 kilobases 5′ of the c-*myc* gene, inside the HL60 amplification unit surrounding and including the c-*myc* gene. Additionally, steady-state levels of c-myc mRNA are elevated in HeLa and C4-I cells relative to other cervical carcinoma cell lines. Thus, in at least some genital tumors, cis-activation of cellular oncogenes by HPV may be involved in malignant transformation of cervical cells.**

The DNA of human papillomavirus (HPV) types 16 and 18 has been found closely associated with human genital cancers (1, 2), supporting the idea that members of this virus group play a key role in the etiology of genital cancer (3). HPV 16 and HPV 18 DNA sequences were also detected in cell lines derived from cervical cancer (2, 4–6). The cell lines HeLa and SW756 contain HPV 18 DNA amplified about 10- to 50-fold together with the flanking cellular sequences, whereas SiHa and C4-I cells contain a single integrated copy of HPV 16 and 18 DNA, respectively (4–8). In these cell lines and in most of the HPV-positive genital carcinomas, one of the opening points for viral integration is in the early region of the viral genome E1/E2 (4, 8). As a consequence, only the E6, E7, and part of the E1 open reading frame and the adjacent cellular sequences can be transcribed from the early viral promoter. The analysis of cDNA clones derived from the HPV 18-containing cell lines HeLa, C4-I, and SW 756 has shown that most, if not all, HPV-positive mRNAs are virus–cell fusion transcripts. The host cell sequences that are spliced to the 5′ HPV 18 exons are different for each cell line (9). The mechanisms by which integration of HPV contributes to the malignant phenotype of the cell are not understood. Previous studies have shown that there are no specific sites for viral integration but the possibility of integration into a specific chromosomal domain could not be excluded (8, 10). We have therefore used the cloned cellular sequences that flank HPV DNA or the 3′ terminal cellular sequences of cDNA clones to determine, by analysis of rodent–human hybrids retaining defined subsets of human chromosomes, the location of the integration sites on the human gene map.

We have found integration sites on chromosomes 3 and 20 in a tumor that harbored multiple HPV 16 integration sites. Integration sites in four cervical carcinoma-derived cell lines were in three different chromosomes, 8, 12, and 13.

MATERIALS AND METHODS

Integration Site Probes. 1.2A and 3.2A represent virus/cell junction fragments cloned from a HPV 16-positive cervical carcinoma that harbors multiple integration sites (7, 8). For hybridization purposes, a 3.4-kilobase (kb) *Hin*dIII unique cellular fragment (3′ of the viral integration site) was subcloned from 3.2A. A 3.8-kb *Bam*HI/*Xba* I fragment represents the unique cellular region of clone 1.2A.

The probes C33.1 (2.0-kb *Bgl* II/*Eco*RI fragment) and 7.16.1 (1.3-kb *Hph* I/*Eco*RI fragment) represent the 3′ terminal host cell sequences of two cDNA clones originally derived from the cervical carcinoma cell lines C4-I and SW756, respectively (9).

H4.1 was subcloned from the genomic clone H4 (11), which encompasses one of the HPV 18 genomes together with the flanking cellular sequences of HeLa cells. H4.1 represents a 1.8-kb *Hin*dIII/*Xba* I fragment situated immediately upstream of HPV 18.

Probe p111/17 (0.4-kb *Eco*RI/*Hin*dIII fragment) represents the 3′ flanking cellular sequence of the integrated HPV 16 genome in the SiHa cell line (M.D., unpublished work).

DNA Probes Used as Markers for Specific Chromosome Regions. DNA probes for each human chromosome were used to characterize the rodent–human hybrid panel (12–16), and sometimes several probes for a specific chromosome region of interest were used: for chromosome 8, c-*myc* (*MYC* in human gene nomenclature) at 8q24 (17) and c-*mos* at 8q11 (18, 19); for chromosome 11, β-globin at 11p15 (20) and Bcl-1 at 11q13 (21); for chromosome 13, three anonymous DNA clones, p7F12 at 13q13, p9D11 at 13q21, and p9A7 at 13q32 (22); for chromosome 12, T4 at 12p12→pter (23), Ki-*ras2* (24), and the C8 homeo box containing gene at 12q12 (25); for chromosome 20, c-*src*-1 at 20q13 (26, 27); for chromosome 3, c-*raf*-1 at 3p25 (28).

All probes were radiolabeled by nick-translation using α-^{32}P-labeled dNTPs to a specific activity of $\approx 1 \times 10^8$ cpm/0.1 μg.

Cells. Isolation, propagation, and characterization of parental cells and somatic cell hybrids have been described (12–17, 23, 25).

For regional localization of HPV integration sites, hybrid cells carrying chromosomal translocations were exploited. For chromosome 20, a series of hybrids derived from a patient (patient 4; ref. 29) with a t(9;22) were described (29);

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Abbreviations: HPV, human papillomavirus; PBL, peripheral blood lymphocytes.

this patient also carried a t(14;20)(14q11→14qter::20pter→20q13), and hybrid cells with the 20q+ chromosome (containing the region 20pter→20q13) were identified (ref. 29; and J. Finan, J. Erikson, C.M.C., and P. Nowell, unpublished results). Hybrid AB3 (see Fig. 1) retained the 20q+ (20pter→20q13::14q11→14qter) and not the 14q−. For regional localization on chromosome 13, we used hybrid AC5 containing a 13q− (13pter→13q14; 7q35→7qter) in the absence of 13q14→13qter and normal 13 (P. Nowell, J. Finan, F. Haluska, and C.M.C., unpublished).

For regional localization on chromosome 8, parental cells HL60 and Colo 320 were used since they contain regions of DNA in the vicinity of the c-*myc* gene at 8q24 that are amplified >15-fold for *myc* and surrounding sequences (30–32). Hybrid cells retaining translocated regions of chromosome 8 were also used. M442S (17) retains a 14q+ (14pter→14q32::8q24→8qter) of a t(8;14) from an African Burkitt lymphoma, P3HR1. The chromosome 8 breakpoint is at least 35 kb 5′ of the first c-*myc* exon (33); thus, the 5′ sequences, c-*myc*, and the rest of chromosome 8 down to 8qter are translocated to the 14q+ chromosome. Additional hybrids of the JI series (34) retain t(2;8) chromosomes retaining defined regions of chromosome 8, and hybrid BC10 retains an 8q+ (8pter→8q24::14q11→14qter) from a T-cell leukemia (35).

Southern Blot Analysis. Cellular DNAs were isolated as described (12), digested with an excess of appropriate restriction enzymes, sized in 0.8% agarose gels, and transferred to nitrocellulose filters as described by Southern (36). Hybridization took place in 50% formamide, 0.6 M NaCl/0.06 M sodium citrate, pH 7.0, 0.2 mg of sonicated salmon sperm DNA per ml, and 1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) at 42°C for 15 hr. After hybridization, filters were washed and exposed to Kodak XAR-5 film with intensifying screens.

RNA Transfer Blot Analysis. Cell lines originating from cervical cancer biopsies were obtained from the American Type Culture Collection or from M. Herlyn (Wistar Institute; SW756). Cytoplasmic RNA was prepared from HeLa, SW756, C4-I, CaSki, SiHa, and C33A cell lines as described elsewhere (37), fractionated in 0.8% formaldehyde/agarose gels, transferred onto a nitrocellulose filter, and hybridized as described for Southern blot analysis.

RESULTS

Chromosomal Localization of Two HPV 16 Integration Sites, 1.2A and 3.2A, Cloned from a Cervical Carcinoma Harboring Multiple Integration Sites. In initial screening of hybrid DNAs for the presence of the probe 1.2A, a correlation with hybrids retaining human chromosome 20 was apparent (Fig. 1, compare column for chromosome 20 with column for 1.2A). Fig. 2 illustrates the Southern blot that allowed assignment of the 1.2A integration site of HPV 16 in the tumor to chromosome region 20pter→20q13; lane 2, Fig. 2, which is positive for 1.2A sequences, contains DNA from hybrid AB3 retaining a t(14;20)(14q11→14qter::20pter→20q13) and human chromosomes 6 and X. A human homolog of v-*src*, c-*src1* (26), has been assigned to human chromosome region 20q13 (27) and, indeed, c-*src1* cosegregates with the 1.2A probe in the hybrid panel (data not shown).

Using the same strategy, the sequences detected by probe 3.2A were localized to human chromosome 3 (see Fig. 1, compare column for chromosome 3 with column on right for 3.2A). A Southern blot after hybridization with the 3.2A probe is shown in Fig. 3; lane 7 contains DNA from a hybrid, PB5, that does not retain the 3.2A integration site; this hybrid does, however, retain c-*raf1* sequences (data not shown), which are at 3p25 (28). Therefore, the 3.2A integration site is not closely linked to c-*raf1* and is probably not located on region 3p25-3pter. Thus, the two integration sites from the tumor are localized at regions 20pter→20q13, possibly near c-*src*-1, and 3p25→3qter, probably not near c-*raf1*.

HPV 18 Integration Site in SW756 Cell Line. The probe 7.16.1 for the integration site of HPV 18 in SW756 cells correlated with the presence of chromosome 12 in the hybrid panel (see Figs. 1 and 4); more specifically, we have hybridized the blots containing hybrid DNAs to three genes that have already been mapped to chromosome 12: the T4 gene at 12p12→pter (23); a homeo box gene at 12q12→12q13 (25); a Ki-*ras2* probe that has been variously mapped to the short arm of 12 (38, 39), the long arm of 12 (24), and both arms of

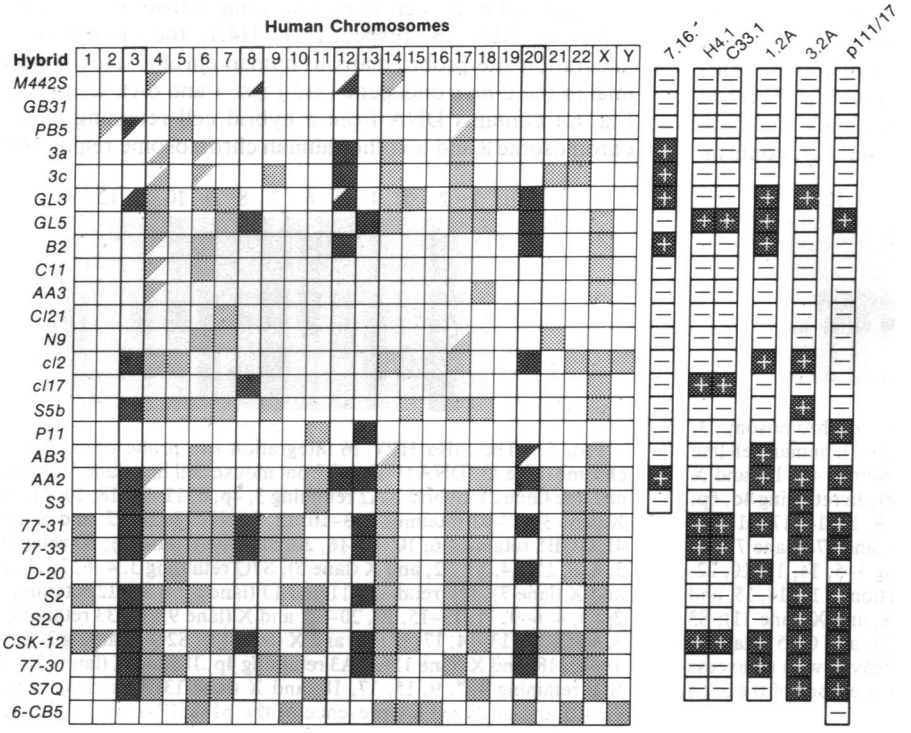

FIG. 1. Presence of HPV cloned integration sites in a panel of rodent–human hybrids: A summary figure showing human chromosomes (listed across the top) present in a panel of rodent–human hybrids (listed down the left side). Presence (▣) or absence (⊟) of each of the cloned probes representing HPV integration sites in the hybrid cells is indicated in the six columns on the right. Stippled boxes in columns under chromosomes indicate retention of an entire chromosome (▨), long arm only (◪), short arm only (▨), or absence of the chromosome (□) in the hybrid clone named on the left. Columns for chromosomes 3, 8, 12, 13, and 20 are boldly outlined and stippled to highlight the correlation of pattern of segregation of the integration site probes (3.2A, H4.1, C33.1, 7.16.1, p111/17, and 1.2A, respectively) with the pattern of retention of these specific chromosomes.

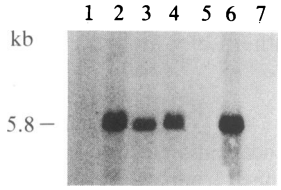

FIG. 2. HPV integration site 1.2A maps to chromosome region 20pter→20q13. DNA (≈10 μg per lane) from hybrid AA3 retaining human chromosomes 4p, 18, and X (lane 1); AB3 retaining 6, 14q11→14qter, 20pter→20q13, and X (lane 2); AA2 retaining 3, 4p, 11–14, 18, 20, 21, and X (lane 3); B2 retaining 4p, 6, 12, 20, and X (lane 4); C11 retaining 4p, 6, and X (lane 5); human peripheral blood lymphocytes (PBL) (lane 6); and mouse cell line (lane 7) was cleaved with an excess of restriction enzyme *Eco*RI, fractionated on an agarose gel, transferred to nitrocellulose filter, and hybridized to radiolabeled 1.2A DNA. Approximate size of the human specific fragment detected is given on the left.

12 (40). We found that this integration site is not closely linked to any of the three markers. Thus, 7.16.1 is on chromosome 12 but not at 12p12→12pter; it is not closely linked to Ki-*ras2* or to the C8 homeo box gene at chromosome region 12q12→12q13.

HPV 16 Integration Site in SiHa Cell Line. The cloned integration site p111/17 from SiHa, a cervical carcinoma cell line with a single integrated HPV 16 genome, did not segregate in the hybrid panel in 100% concordance with any human chromosome region (Figs. 1 and 5). The best candidate for location of the p111/17 integration site was chromosome 13 with 2 discordancies (2 of 28 tested or 7%), with chromosome 11 the second best candidate with 5 discordancies (18%) (see Fig. 1, compare column for p111/17 with columns for chromosomes 13 and 11).

Convincing evidence that p111/17 maps to chromosome 13 was obtained by hybridizing the probe to DNA from a pair of hybrids (41), c113 and a 6-thioguanine back-selected subclone of c113. The single intact human chromosome present in hybrid c113 is a derivative 11 translocation chromosome (11pter→11q23::Xq25-26→Xqter); there is also a mouse chromosome containing a fragment of a human chromosome identified by G11 staining. The 6-thioguanine back-selected subclone had lost the derivative 11 chromosome but retained the mouse chromosome containing a human fragment (41). Restriction enzyme-digested DNA from both of these hybrids retains two markers for chromosome 13 at 13q13 (p7F12) and 13q21 (p9D11) (but not the P9A7 marker at 13q32), and both hybrids retain the p111/17 sequences (not shown), confirming the location of p111/17 sequences at 13pter→13q32. To further pinpoint this integration site on chromosome 13, we took advantage of a hybrid clone carrying a t(7;13)(q35;q14) chromosome. This hybrid, AC5, retained the 13q− chromosome but not a normal 13 or the region 13q14→13qter. Restriction enzyme-cleaved AC5 DNA retains a chromosome 13-specific anonymous DNA fragment, p7F12, at 13q12→13q13 but does not retain two more distal chromosome 13 markers, p9D11 [at 13q21 (22)] and p9A7 [at 13q32→13q33 (22)] (F. Haluska and C.M.C., unpublished). Probe p111/17 sequences were not detected in *Hin*dIII-digested AC5 DNA (not shown), indicating that the gene detected by the p111/17 probe is distal to region 13q14.

Thus, p111/17 maps to 13q14→13q31, in agreement with the finding that HPV 16 is integrated at 13q21-31 in SiHa cells (A. Mincheva, L.G., and H. zur Hausen, unpublished work).

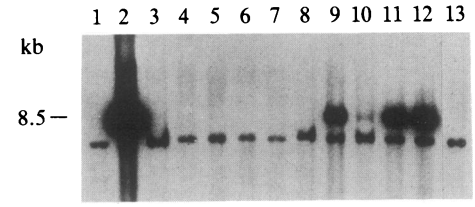

FIG. 3. HPV 16 integration site 3.2A maps to chromosome 3. DNA (≈10 μg per lane) from mouse cell line (lane 1); human cell line (lane 2); hybrid AA3 retaining human chromosomes 4p, 18, and X (lane 3); B2 retaining 4p, 6, 12, 20, and X (lane 4); 3a retaining 4q, 6p, 12, 14, 17, and 22 (lane 5); 3c retaining 4q, 6p, 9, 12, 14, 17, 21, and 22 (lane 6); PBS retaining 2p, a portion of 3p, 5, and 17q (lane 7); N9 retaining 6, 7, 17q, and 21 (lane 8); cl2 retaining 3–5, 14, 17, 20, 22, X, and Y (lane 9); GL3 retaining 3, 4, 6, 7, a portion of 12, 14, 15, and 17–20 (lane 10); S5b retaining 3–7, 9, 15, 17, 18, and X (lane 11); S3 retaining 3, 4, 6, 11, 14, 17, 22, and X (lane 12); and GL5 retaining 4, 7, 8, 13, 14, 17, 18, 20, and X (lane 13) was cleaved with an excess of restriction enzyme *Bam*HI and analyzed for presence of the 3.2A locus.

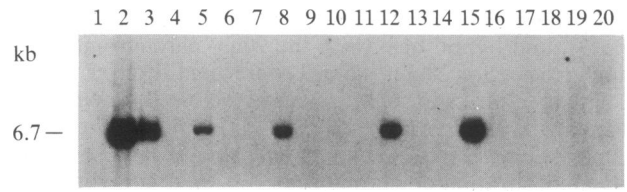

FIG. 4. HPV 18 integration site 7.16.1 from SW756 cells is located on chromosome 12. DNA (≈10 μg per lane) from mouse cell line (lane 1); human cell line (lane 2); hybrid 3a retaining 4q, 6p, 12, 14, 17, and 22 (lane 3); S5b retaining 3–7, 9, 15, 17, 18, and X (lane 4); AA2 retaining 3, 4p, 6, 11–14, 18, 20, 21, and X (lane 5); M442S retaining 4p, 8q24-8qter, 12q, and 14pter→14q32 (lane 6); GB31 retaining 17 (lane 7); GL3 retaining 3, 4, 6, 7, 12 (but missing the T4 gene at 12p12-pter), 14, 15, and 17–20 (lane 8); N9 retaining 6, 7, 17q, and 21 (lane 9); cl21 retaining 7 (lane 10); AA3 retaining 4p, 18, and X (lane 11); B2 retaining 4p, 6, 12, 20, and X (lane 12); C11 retaining 4p, 6, and X (lane 13); PB5 retaining 2p, a portion of 3p, 5, and 17q (lane 14); 3c retaining 4q, 6p, 9, 12, 14, 17, 21, and 22 (lane 15); S3 retaining 3, 4, 6, 11, 14, 17, 22, and X (lane 16); cl17 retaining chromosome 8 (lane 17); P11 retaining 11 and 13 (lane 18); GL5 retaining 4, 7, 8, 13, 14, 17, 18, 20, and X (lane 19); and marker (lane 20) was cleaved with *Eco*RI and analyzed for presence of the 7.16.1 locus.

Localization of HPV 18 Integration Sites in C4-I and HeLa Cells to Chromosome 8. C4-I and HeLa cells are cervical carcinoma-derived cell lines harboring single and multiple copies of HPV 18, respectively. H4.1, the cloned HeLa integration site probe, maps to human chromosome 8 proximal to the c-*myc* oncogene (see Figs. 1 and 6*A*). Lane 10 of Fig. 6*A* contains DNA from a hybrid cell retaining human chromosome 8 and no other human chromosome region (42);

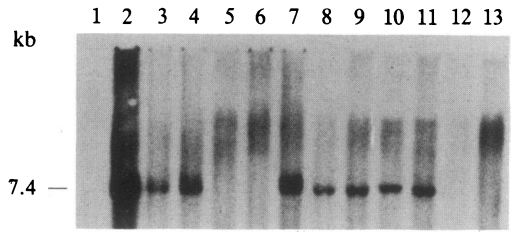

FIG. 5. The SiHa HPV 16 integration site probe p111/17 is on chromosome 13. DNA (≈10 μg) from mouse cell line (lane 1); human cell line (lane 2); hybrid AA2 retaining 3, 4p, 6, 11–14, 18, 20, 21, and X (lane 3); 77–31 retaining 1, 3–10, 13, 14, 17, 18, 20, 22, and X (lane 4); 6-CB5 retaining 6, 10, 14–16, 20, 22, and Y (lane 5); S3 retaining 3, 4, 6, 11, 14, 17, 22, and X (lane 6); S7Q retaining 3, 4, 6, 7, 11, 18, and X (lane 7); 11p retaining 11 and 13 (lane 8); CSK-12 retaining 1, 2p, 3, 4, 6–9, 11, 13–15, 18, 20–22, and X (lane 9); 77–33 retaining 1, 3, 4p, 5–10, 13, 14, 17, 18, 22, and X (lane 10); S2Q retaining 3, 4, 11, 14, 17, 18, and X (lane 11); AA3 retaining 4p, 18, and X (lane 12); and S5b retaining 3–7, 9, 15, 17, 18, and X (lane 13) was cleaved with *Eco*RI and analyzed for presence of the p111/17 locus.

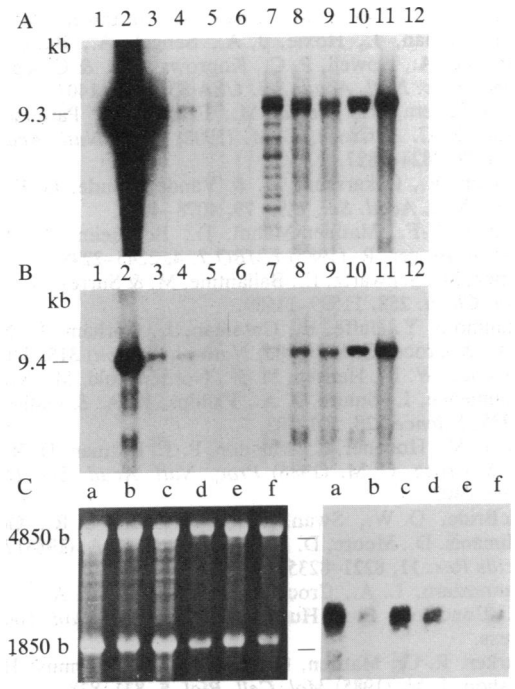

FIG. 6. HPV 18 integration in chromosome 8. (*A*) The HeLa HPV 18 integration site probe H4.1 is on chromosome 8, 5′ of c-*myc* and within the HL60 c-*myc* amplification unit. DNA (≈10 μg per lane) from mouse cell line (lane 1); human cell line HL60 (lane 2); hybrid CSK-12 retaining human chromosomes 1, 2p, 3, 4, 6–9, 11, 13–15, 18, 20–22, and X (lane 3); GL5 retaining 4, 7, 8, 13, 14, 17, 18, 20, and X (lane 4); cl2 retaining 3–5, 14, 17, 20, 22, X, and Y (lane 5); M442S retaining 4p, 8q24→8qter (from 5′ of c-*myc* to 8qter), 12q, and 14pter→14q32 (lane 6); 77-33 retaining 1, 3, 4p, 5–10, 13, 14, 17, 18, 22, and X (lane 7); hybrid J14-2 (not in Fig. 1) retaining an 8q+ chromosome [(8pter→8q24::2p11→2pter) (ref. 34) in which the break on chromosome 8 is ≈30 kb 3′ of the c-*myc* gene (ref. 34) leaving c-*myc* and ≈30 kb of 3′ sequence on this 8q+ chromosome] in the absence of a normal 8 and the 2p− chromosome [(2p11→2qter::8q24→8qter) (ref. 34) (lane 8); hybrid J15-4 (not in Fig. 1) retaining a normal chromosome 8 and a 2p− chromosome in the absence of the 8q+ chromosome (ref. 34) (lane 9); cl17 retaining human chromosome 8 (lane 10); hybrid BC10 retaining a 8q+ [(8pter→8q24::14q11→14qter) in which the break in chromosome 8 is >30 kb 3′ of the c-*myc* gene leaving c-*myc* on the 8q+ chromosome (ref. 35)] in the absence of a normal chromosome 8 and the 14q− chromosome that carries the 8q24→8qter sequences (lane 11); and Chinese hamster (lane 12) was digested with restriction enzyme *Eco*RI and analyzed for presence of the H4.1 sequences. Lane 7 contains DNA from a hybrid, 77-33, isolated from fusion of HL60 cells with mouse myeloma cells; this hybrid retains the 8q+ (with abnormal banding region and amplified c-*myc*) from HL60 (43) in <20% of hybrid cells. (*B*) HPV 18 integration site from C4-I cells, probe C33.1, maps to chromosome 8pter→8q24, 5′ of the c-*myc* gene. The same blot depicted in *A* was stripped of the H4.1 probe and hybridized to the C33.1 probe. C33.1 probe is present in each lane in which H4.1 was present and is absent in the same lanes that were missing H4.1 sequences. The C33.1 probe is not amplified in HL60 (lane 3) or in hybrid 77-33 (lane 7), which retains an 8q+ with abnormal banding from HL60 cells. Compare with lane 7 of *A*. (*C*) Expression of c-*myc* in cell lines derived from cervical carcinoma by RNA transfer blot analysis. Ten micrograms of cytoplasmic RNA of each cell line was separated in lanes a–f (HeLa, SW756, C4-I, CaSki, SiHa, and C33A, respectively). b, Bases. Approximately equal amounts of total RNA are visible before transfer of RNA onto the filter membrane (*Left*). The filter was hybridized with the third exon of c-*myc* as probe and autoradiographed (*Right*).

this hybrid retains H4.1 sequences. Fig. 6*A* also illustrates the regional localization of H4.1 proximal to the c-*myc* gene at 8q24. Hybrid M442S in lane 6 contains DNA from a hybrid retaining a 14q+ chromosome (8q24→8qter::14pter→14q32) from P3HR1 Burkitt lymphoma cells (17). This 14q+ chromosome contains the region of chromosome 8 extending from the breakpoint at ≈30 kilobase pairs 5′ of c-*myc* to the terminus of the long arm (8q24→8qter) (17, 23) and is negative for the H4.1 probe (Fig. 6*A*, lane 6). Conversely, hybrids retaining normal chromosome 8 (Fig. 6*A*, lanes 4, 7, 9, and 10) or the region 8pter→8q24 (Fig. 6*A*, lanes 8 and 11) extending from the tip of the short arm of chromosome 8 to a region 3′ of the c-*myc* gene (34, 35) are positive for the H4.1 sequences. Thus, segregation of the H4.1 sequences in the hybrids retaining translocated regions of chromosome 8 allows assignment of this integration site on chromosome 8, 5′ of the c-*myc* gene. On the same blot (Fig. 6*A*, lane 2) the human control is HL60 DNA, which has a region of ≈90 kb surrounding and including the c-*myc* gene, which is amplified ≈16-fold (30, 31). Since we know that the amplification unit extends at least 50 kb on the 3′ side of c-*myc* in HL60 (44) and the entire unit of amplification is ≈90 kb (45) and the H4.1 probe is clearly amplified in HL60 (see lane 2, Fig. 6*A*), this narrows the integration site of H4.1 to somewhere within the ≈30–40 kb 5′ of the c-*myc* gene (assuming the amplification unit is not discontinuous); H4.1 sequences are also amplified (not shown) in Colo 320, another tumor containing an amplified c-*myc* region (32).

The HPV 18 integration site probe C33.1 from C4-I cells segregates in our hybrid panel 100% concordantly (Fig. 6*B*) with the H4.1 probe (see Fig. 1 and compare Fig. 6 *A* and *B*) and is thus also on chromosome 8 proximal to c-*myc* (i.e., between 8pter→8q24, 5′ of c-*myc*) but is not amplified in HL60 (Fig. 6*B*, lane 2) or Colo 320 (not shown). Thus, at least one, and possibly two of four cervical carcinoma cell lines contain HPV genomes integrated upstream of c-*myc*.

Expression of the c-*myc* Gene in Cervical Cancer-Derived Cell Lines. To determine if the HPV genomes in HeLa and C4-I cells might exert a cis-acting influence on expression of the c-*myc* gene, we have compared steady-state levels of c-myc mRNA in cervical carcinoma-derived cell lines by RNA transfer blot analysis. As depicted in Fig. 6*C*, HeLa and C4-I cell lines (Fig. 6*C Right*, lanes a and c, respectively) do express higher steady-state levels of c-myc mRNA than do SW756 and SiHa (Fig. 6*C Right*, lanes b and e, respectively). No HPV sequences in C33A cells (Fig. 6*C*, lane f) have been identified. It is, however, of interest that in Caski cells, clusters of HPV genomes have been observed by *in situ* hybridization (A. Mincheva, L.G., and H. zur Hausen, unpublished work) on chromosomes 2, 7, 11, 12, 20, and 21 and several marker chromosomes; one of the marker chromosomes exhibiting a HPV cluster was identified as a t(X;8)(qter→q11; q11→qter) on which the label was near the terminal part of the 8q region. Thus, Caski cells may have three integration sites (on chromosomes 12, 20, and 8) in common with those we have identified; Caski cells do show a higher level of c-myc mRNA (Fig. 6*C*, lanes d) than do SW756 and SiHa cells. In summary, there is a higher level of c-*myc* expression in cervical carcinoma cell lines containing HPV integration sites near c-*myc* relative to those that do not.

DISCUSSION

The mechanism by which HPV genomes, which are associated with genital tumors (1, 2), contribute to the malignant process is not known. It could be that these genomes, some regions of which are consistently transcribed in cervical carcinoma-derived cell lines and some genital tumors (4), code for trans-acting factors that activate cellular genes or pathways that lead directly or indirectly to the malignant phenotype. Alternatively, or additionally, papilloma sequences could act as insertional cis-acting promoter/enhancer mutagens that could activate nearby cellular protooncogenes. We offer evidence that, at least in some cases, the

papillomavirus DNA is integrated in regions of the human genome that are near cellular protooncogenes. The HPV 18 integration site in HeLa cells is closely 5′ of the c-*myc* locus, and in C4-I cells the HPV 18 integration site is on chromosome 8, 5′ of c-*myc*, but the precise location is not yet known. In both of these cell lines, c-*myc* expression is elevated relative to SiHa and SW756, which do not exhibit HPV integration on chromosome 8. These results are consistent with the hypothesis that HPV genomes contribute to malignancy by cis-activation of nearby cellular oncogenes. It will now be necessary to determine how close the HPV 18 sequences in C4-I cells are to c-*myc* and to determine if the integration sites in chromosomes 12, 13, and 20 are near known or putative oncogenes that are, concordantly with those integration sites, expressed at high levels. Further fine mapping of HPV integration sites and study of expression of nearby, possibly activated, genes will aid in understanding the role of HPV in human malignancy.

Note Added in Proof. We recently learned that Popescu *et al.* (46) will report the localization of HPV 18 DNA sequences in HeLa cells to chromosomes 5, 8, 9, and 22.

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