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Transformation of Human Cells by Oncogenic Viruses Supports Permissiveness for Parvovirus H-1 Propagation

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Parvovirus H-1 has been shown to suppress spontaneous and chemically or virally induced tumorigenesis in hamsters. In human cell culture systems propagation of H-1 is restricted to transformed cells, which are killed by H-1 infection, in contrast to normal diploid cells, which are nonpermissive for H-1. By analyzing the permissiveness of a variety of human cells for H-1, it was determined that the majority of tested transformed or immortalized cells which were permissive for H-1 contained the DNA of oncogenic viruses (human papillomavirus, simian virus 40, adenovirus, hepatitis B virus, Epstein-Barr virus, and human T-cell lymphotropic virus type I). Of six transformed cell lines negative for persisting tumor virus DNA, only two were permissive for H-1, while two were semipermissive and two were nonpermissive. Thus, persistence and expression of tumor virus functions appear to promote full permissiveness for H-1 in human cells. However, neither expression of genes of specific viral genomes nor the transformed state of apparently virus-free cells alone was sufficient to render human cells permissive for H-1. Therefore, the effect of tumor virus functions on H-1 in transformed cells seems to be indirect, probably mediated by cellular factors which are induced or switched off during the transformation process. It appears that similar factors are induced or switched off by 5-azacytidine or calcium phosphate, both known inducers of cellular gene expression.

Mammalian parvoviruses are divided into two groups, the autonomous parvoviruses and the dependoviruses (adeno-associated viruses). There is recent evidence that suggests that this taxonomy does not reflect the properties of parvovirus replication. Under certain conditions dependoviruses have been shown to replicate without helper viruses (23, 24, 31, 32), whereas the autonomous parvoviruses H-1 and minute virus of mice could be induced in nonpermissive human cells by transformation with simian virus 40 (4, 6).

Parvovirus H-1 is a small DNA virus which replicates in the nucleus of infected cells and apparently depends on host cell functions for its own replication (18). It contains a linear single-stranded 5.17-kilobase (kb) genome with two overlapping transcription units and two promoters at map positions 4 and 38 (20). The early promoter, P4, regulates the expression of the two noncapsid proteins, NS1 and NS2; the late promoter, P38, regulates the expression of the two capsid proteins, VP1 and VP2.

Parvovirus H-1 is capable of efficiently suppressing spontaneous and chemically or virally induced carcinogenesis in hamsters in vivo: the incidence of spontaneous tumors is reduced from 5 to 0.23% (27), of adenovirus type 12-induced solid tumors from 67 to 28% (29), and of 7,12-dimethylbenz(a)anthracene-induced tumors from 95 to 38% (30) by prior infection with H-1.

The mechanism of tumor suppression by parvoviruses is not understood. A possible insight into this mechanism is that transformed rodent and human cells in vitro show an enhanced susceptibility to parvovirus-induced cell killing (5). This implies that H-1 virus proliferates not in normal but in transformed human cells (28), which are subsequently killed during parvovirus replication (4). The killing appears to be mediated by expression of the parvovirus noncapsid proteins NS1 and NS2 in H-1-permissive cells (19). Since the expression of these proteins is regulated by the H-1 early promoter, cells not able to support the complete replicative cycle of H-1 may be killed after H-1 infection.

Here we ask whether the transformation event renders human cells permissive for H-1. Spontaneous and chemically induced transformation of human cells increases the susceptibility to H-1-induced cell killing but is not sufficient for permissiveness. We provide evidence that a helper function for the replicative cycle of the autonomous parvovirus H-1 in transformed cells is mediated by a common cellular mechanism, which may be supported by specific types of papillomaviruses, adenoviruses, hepatitis B virus, Epstein-Barr virus, and retroviruses persisting in the same cells.

Permissive human cells seem to express cellular H-1 activating factor(s), which are induced by oncogenic viruses in the course of the transformation event. It appears that at least some of these factors are also induced by 5-azacytidine and calcium phosphate, both known to induce cellular genes (11, 16).

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used: Ad5 and Ad12, adenovirus types 5 and 12, respectively; mAMSA, 4′-(9-acridinylamino)-methanesulfon-*m*-aniside; CPE, cytopathic effect; DMBA, 7,12-dimethylbenz(a)anthracene; DMSO, dimethyl sulfoxide; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HEFs, primary human embryonal fibroblasts; HTLV, human T-cell lymphotropic virus; HPV, human papillomavirus; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NC, nitrocellulose; p.i., postinfection; SDS, sodium dodecyl sulfate; SV40, simian virus 40.

Cell lines and virus stocks. SV40-transformed newborn human kidney cells (NB-E; 25), HeLa cells, HeLa × human fibroblast hybrid cells (444, nontumorigenic; CGL3, tumorigenic) (26), and human diploid fibroblasts were grown in Eagle minimal essential medium (Boehringer GmbH, Mannheim, Federal Republic of Germany).

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Spontaneously immortalized human keratinocytes (HaCaT) (1), cervical carcinoma cell lines SiHa and Caski, a subclone of the vulvar carcinoma cell line A431 (A431-Cl3), Ad5 E1a gene-transformed human fibroblasts (293 cells), SV40-transformed human fibroblasts (SV80), HBV-containing human liver cells (PLC/PRF/5 line), simian cell lines CV-1 and Vero, and SV40-transformed simian cell line Cos were cultivated in Dulbecco modified essential medium (Boehringer).

Human foreskin keratinocytes and human foreskin keratinocytes immortalized by transfection with HPV type 16 (HPV-16) DNA (HPK I and HPK II) (9) were cultivated in FAD medium (9). The Burkitt's lymphoma cell lines BJA-B (15), BL2, and BL72, the adult T-cell leukemia cell lines MT-1, MT-4, Jurkat, and Molt-4, the EBV (B95-8)-immortalized human B-lymphocyte cell lines LCL-Gi (H. zur Hausen, unpublished data) and LCL-IARC 277 were cultivated in RMPI 1640 medium.

All media were supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum (Boehringer), penicillin (100 U/ml; Boehringer), streptomycin (100 μg/ml; Boehringer), and L-glutamine (1 mM; Boehringer). All cell lines were cultivated in a water-saturated CO_2 atmosphere (5%) at 37°C.

Wild-type H-1 virus was propagated in NB-E cells. Stock virus was prepared from the cleared supernatant of freezed-thawed (three times) NB-E cells when the CPE was complete, yielding approximately 3×10^7 PFU/ml. This stock virus was confirmed to be devoid of infectious SV40 by hybridization with ^{32}P-labeled SV40 DNA (21) of a Southern blot of H-1-infected CV-1 cells, which are permissive for SV40. Ad12 was propagated in HeLa cells.

Analysis of H-1 DNA replication. H-1 DNA replication was measured by applying 0.1 to 1.0 μg of cellular DNA onto NC filters, using a Minifold II apparatus (Schleicher & Schuell, Dassel, Federal Republic of Germany). The DNA of the H-1-infected cells was extracted with phenol-chloroform at different times p.i. By hybridization of duplicate filters with ^{32}P-labeled human β-albumin DNA (21) as a marker for the amount of DNA applied and with ^{32}P-labeled cloned H-1 DNA (pSR1), the ratio of H-1 DNA to cellular DNA was determined (2). The ratio of H-1 DNA replication or amplification corresponds to the ratio of the cell-associated viral DNA to input viral DNA as measured in cells infected with H-1 30 min p.i.

To identify the replicative forms of H-1 DNA, Southern blot analysis was performed with phenol-chloroform-extracted DNA from H-1-infected cells cleaved with *Bam*HI and *Bgl*I, which are noncutting enzymes with regard to H-1 DNA.

Virus titration. For titration of infectious virus a modified plaque assay was used. Cells were harvested 4 days p.i. and subjected to three freeze-thaw cycles. The cleared supernatants were diluted in minimal essential medium. About 7×10^5 to 8×10^5 indicator NB-E cells seeded on 60-mm petri dishes were inoculated with these diluted supernatants (0.4 ml per petri dish) at 37°C in a CO_2 incubator. After 24 h, when every infected NB-E cell had approximately 1,000 copies of the H-1 genome, the cells were transferred in situ onto an NC filter by applying a filter directly onto the monolayer after removal of the medium (at this stage of infection new virions are not yet released in the supernatant). The DNA of the transferred cells was denaturated by placing a Whatman 3MM filter paper saturated with 0.5 M NaOH–1.5 M NaCl for 5 to 10 min on the NC filters, followed by neutralization with a 3MM filter paper saturated with 1 M Tris hydrochloride (pH 7.0)–20× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate). After hybridization with ^{32}P-labeled H-1 DNA (21) a single infected cell area results in a single signal after exposure, corresponding to a plaque of a plaque assay. This has been confirmed in parallel conventional plaque assays using agar overlay and neutral red staining of surviving cells.

Analysis of H-1 RNA. Extraction of cytoplasmic RNA of H-1-infected cells 3 days p.i. was performed as described by de Villiers and Schaffner (7). Total cytoplasmic RNA was electrophoresed in 1% agarose gels and blotted on NC filters (Schleicher & Schuell).

Determination of H-1-induced cell killing. The growth kinetics of uninfected and infected cell cultures were compared. Total cell numbers and cell viability were determined by the trypan blue dye exclusion test.

Calcium phosphate-mediated transfection. DNA transfection was performed by the $CaPO_4$-mediated transfection protocol of Chen and Okayama (3). Briefly, 20 μg of DNA was precipitated with 0.125 M $CaCl_2$ and 25 mM BES [*N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid]–0.75 mM Na_2HPO_4 (pH 6.95) for 20 min at room temperature. A 1-ml portion of this mixture was added dropwise to the plated cells (grown in 10 ml of minimal essential medium) and incubated for 24 h at 35°C under 3% CO_2.

Chemicals. mAMSA (provided by Warner Lambert Co., Ann Arbor, Mich.), DMBA (Sigma Chemical Co., Deisenhofen, Federal Republic of Germany), 5-azacytidine (Serva, Heidelberg, Federal Republic of Germany), the calcium ionophore A23187 (Serva), and MNNG (Serva) were freshly dissolved in DMSO and used at the concentrations indicated below. The final concentration of DMSO in the medium never exceeded 0.1%.

RESULTS

Screening of human cells for permissiveness for H-1. In initial experiments we tested human primary cells and cells of different human lines for their ability to amplify parvovirus DNA and to produce infectious particles. In all cells tested the viral genome was amplified, showing that H-1 virus could infect all lines.

In three primary cultures of HEFs from different donors and one primary culture of primary human foreskin keratinocytes, the viral DNA was amplified up to 26-fold but failed to produce infectious parvovirus progeny (Table 1). No change of the growth rate could be detected in HEF cultures after parvovirus infection. Two weeks p.i., parvovirus DNA was no longer detectable by slot blot hybridization.

(i) It was observed that all cell lines tested containing DNA of oncogenic viruses were permissive for H-1: HPK I, HPK II, HeLa, SiHa, and Caski, which contain HPV DNA; NB-E and SV80, which contain SV40 DNA; LCL-IARC 277, LCL-Gi and BL72, which contain EBV DNA; MT-1 and MT-4, which contain HTLV type I (HTLV-I) DNA; and 293 and PLC, which contain Ad5 and HBV DNA (Table 1). These cell lines amplified the parvovirus DNA by factors of 9 to 5,100 (measured as the ratio of H-1 DNA 4 days p.i. versus input DNA 0.5 h p.i.). Also, infectious H-1 particles were produced by factors ranging between 2 and 1,550 (measured as the ratio of infectious H-1 particles 4 days p.i. versus input virus 0.5 h p.i.). All of these cells were killed in the course of parvovirus infection (Table 1).

(ii) Four of six cell lines thus far negative for persisting viral DNA were not permissive for H-1. The spontaneously immortalized human keratinocyte cell line HaCaT did not produce

TABLE 1. Permissiveness of different human cell lines for parvovirus H-1[a]

Cell type	Origin[b]	Cell line	Persisting virus	H-1-DNA replication[c]	Production of infectious particles[d]	Cell killing[e]
Human primary	f	Embryonal		4–26	0.3	−
	k	Foreskin		5	0.3	−
Human immortalized	k	HaCaT		53	0.4	+/−
	k	HPKI	HPV-16	5,028	81	+
	k	HPK II	HPV-16	256	30	+
	n	NB-E	SV40	2,940	1,553	+
	f	SV80	SV40	5,186	24	+
	f	293	Ad5-E1a	637	30	+
	b	LCL-IARC 277	EBV	122	3.3	+
	b	LCL-Gi	EBV	9	2	+
Human tumor derived	k	A431		42	0.5	+/−
	b	BJA-B		451	514	+
	b	Molt-4		194	43	+
	b	BL2		677	36	+/−
	b	Jurkat		39	8.6	+/−
	l	PLC	HBV	883	35	+
	k	HeLa	HPV-18	468	243	+
	k	SiHa	HPV-16	1,050	49	+
	k	CaSki	HPV-16	294	5	+
	b	MT-1	HTLV-I	232	71	+
	b	MT-4	HTLV-I	55	1.8	+
	b	BL72	EBV	61	112	+
Simian		CV-1		5	0.5	−
		Vero		20	0.7	−
		Cos	SV40	480	70	+

[a] Human and simian cell lines of various origins were tested for their ability to amplify H-1 DNA and to produce infectious H-1 particles and for their susceptibility to H-1-induced cell killing.

[b] b, Blood cells; f, fibroblasts; k, keratinocytes; l, liver cells; n, kidney cells.

[c] Ratio of the amount of H-1 DNA 4 days p.i. to the amount of H-1 input DNA (0.5 h p.i.).

[d] Ratio of infectious H-1 (titer 4 days p.i.) to input virus (titer 0.5 h p.i.).

[e] +, 3 to 30 days p.i., no living cells detectable; +/−, 1 to 30 days p.i., up to 95% of the cells killed, but resistant cells emerged, yielding cultures of resistant cells; −, no cell killing detectable.

infectious H-1 particles (Table 1) and showed a reduced response to parvovirus-induced cell killing. Forty-eight percent of infected HaCaT cells were killed during the first 2 days p.i., but then infected cultures grew normally (Fig. 1B). Two weeks p.i. parvovirus DNA was no longer detectable in these cultures. Also, H-1-infected cultures of a subclone of the vulvar carcinoma cell line A431 failed to produce infectious H-1 progeny (Table 1), and over 95% of these cells were killed in the course of parvovirus infection, but some resistant cells could be detected. These cells failed to grow and died within 4 weeks. Since HaCaT and A431 cells were not able to produce H-1, they were classified as nonpermissive, although they revealed cytopathic changes.

The T-cell leukemia cell line Jurkat and the Burkitt's lymphoma cell line BL2 also showed a reduced susceptibility to H-1-induced cell killing, although these cells produced infectious particles (Table 1). More than 95% of the cells were killed within 6 days p.i., but 1 to 4 weeks p.i. resistant cells growing within infected cultures could be detected (Fig. 1A) containing parvovirus DNA. A second infection of these cells 4 weeks p.i. did not result in H-1-induced cell killing. Therefore, BL2 and Jurkat cells are classified as semipermissive for H-1.

(iii) Results similar to those obtained with human cells were obtained with simian cells. SV40-immortalized Cos cells were fully permissive for H-1, but spontaneously immortalized parental CV-1 cells and Vero cells, which do not contain detectable viral DNA, were not permissive for H-1.

In all cells tested, parvovirus replicative form I DNA (double-stranded monomeric H-1 DNA) and replicative form II DNA (double-stranded dimeric H-1 DNA) could be detected after infection, indicating that DNA amplification/replication is not the step at which the parvovirus replicative cycle is blocked in primary or other nonpermissive cells (data not shown).

To elucidate whether permissiveness for H-1 is regulated by positive or negative factors, we tested hybrids between the permissive HPV-18-containing cervical carcinoma cell line HeLa and the nonpermissive normal human fibroblasts initially developed by Srivatsan et al. (26). The hybrid cells (line 444) contain HPV-18 DNA but have lost the malignant phenotype. Also CGL3 cells, which are tumorigenic revertants of 444, were tested. HeLa, 444, and CGL3 cells produced almost the same amounts of infectious particles and were killed by parvovirus infection during the first 5 to 6 days p.i. These findings stress the possible role of the presence of HPV DNA within the cell, indicating that at least under conditions of in vitro cultivation the tumorigenic phenotype is not a prerequisite for H-1 permissiveness.

Induction of permissiveness for H-1. Nonpermissive human fibroblasts became permissive after transformation by SV40 or susceptible to H-1-induced cell killing after modification by the chemical carcinogen 4-nitroquinoline 1-oxide or γ irradiation (5, 6). In order to elucidate whether initiation events are sufficient to induce permissiveness for H-1, primary human fibroblasts were treated with chemical or physical carcinogens. Neither the chemical carcinogen MNNG (0.1 to 100 μM), DMBA (0.1 to 1.0 μg/ml), or mAMSA (0.01 to 10.0 μM) nor irradiation (UV light; 0.5 to 60 J m^{-2}) could stimulate replication of H-1 DNA or the production of infectious H-1 particles in a transient assay. This is in agreement with results of others (J. Rommelaere and J. Cornelis, personal communication).

Since virus-induced immortalization is sufficient to render

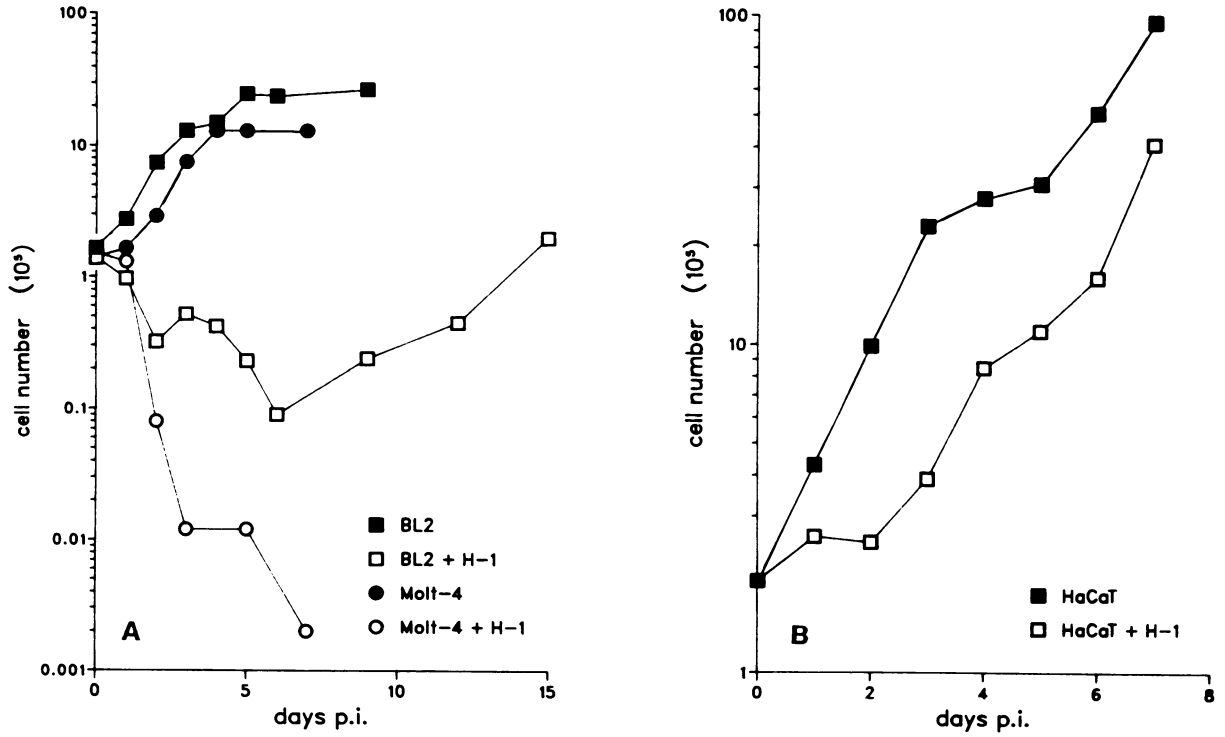

FIG. 1. Growth kinetics of H-1-infected and uninfected human cell lines thus far negative for persisting viral DNA (mean of two independent experiments). (A) Whereas in H-1-infected Molt-4 cultures no cells survived, in infected BL2 cultures more than 95% of the cells were killed but resistant cells emerged. (B) Forty-eight percent of HaCaT cells were killed after H-1 infection during the first 2 days p.i., but the cells showed normal growth rates thereafter.

human cells permissive for H-1, it is suggestive that the presence of oncogenic viruses would exert a helper effect for the complete replicative cycle of H-1. To analyze whether infection with such helper viruses is sufficient for permissiveness for H-1, we infected nonpermissive human cells with SV40 or Ad12 simultaneously with H-1 virus. Whereas permissiveness for H-1 in nonpermissive cells was slightly induced by Ad12 (cf. reference 14), simultaneous infection with SV40 and H-1 did not result in production of infectious H-1 particles (Table 2).

In view of the fact that human cells are semipermissive for SV40, simultaneous infection of spontaneously immortalized simian CV-1 and Vero cells was also performed since these cells are nonpermissive for H-1 but highly susceptible for SV40 production. Even under these conditions infectious H-1 particles were not produced. SV40-transformed simian Cos cells, however, were permissive for H-1, implying that neither SV40 early not late gene expression is sufficient to allow permissiveness for H-1 but that additional factors linked to the virus-induced transformation/immortalization event are required.

In order to test whether 5-azacytidine, a known inducer of demethylation (11), could induce permissiveness, nonpermissive HEF cells were pretreated for 24 h with 2 μM 5-azacytidine. 5-Azacytidine stimulated parvovirus DNA amplification and RNA synthesis. In nontreated HEFs only 4.1- and 1.3-kb transcripts could be detected; treated cells showed additional 2.5- and 3.5-kb transcripts (see Fig. 3).

It is difficult to assign the transcripts found in our experiments to specific genes since only limited and conflicting information about the correspondence of H-1 transcripts and H-1 genes has been reported. Most data on H-1 transcripts resulted from S1 nuclease mapping, yielding transcript sizes in rat cells of 4.7, 3.0, 2.8, 1.45, and 1.3 kb (10) or 4.8, 3.2, and 2.9 kb (13).

In spite of the induction of parvovirus RNA synthesis, no production of infectious particles was detected in 5-azacytidine-treated cells (Table 2). In contrast, treatment of permissive cell lines such as HeLa, CGL3, and 444 with 5-azacytidine resulted in a decrease of H-1 DNA amplification, H-1 RNA expression, and the yield of infectious particles.

It has also been reported that calcium phosphate precipitates transform rodent cells (12) and activate certain cellular

TABLE 2. Induction of permissiveness for H-1 virus

Cell line and treatment	H-1-DNA replication[a]	Production of infectious particles[b]
HEF	21	0.1
+Ad12	65	0.5
+SV40	41	0.1
+5-Azacytidine	167	0.2
+$CaPO_4$	287	0.8
HaCaT	53	0.4
+Ad12	48	1.0
+SV40	52	0.5
A431	42	0.5
+Ad12	1,948	2.3
+SV40	269	0.6

[a] Ratio of the amount of H-1 DNA 4 days p.i. to the amount of H-1 input DNA (0.5 h p.i.).

[b] Ratio of infectious H-1 (titer 4 days p.i.) to input virus (titer 0.5 h p.i.). For further information see the text.

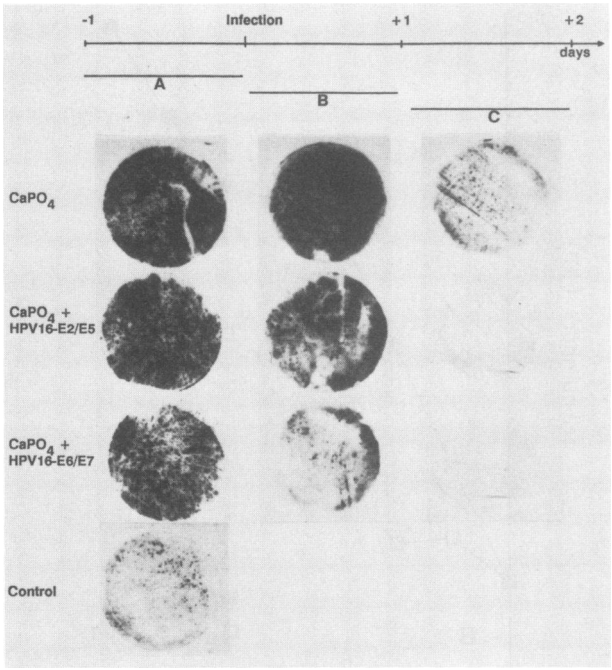

FIG. 2. In situ hybridization of calcium phosphate-treated, H-1-infected HEF cells with ^{32}P-labeled H-1 DNA (21) 4 days p.i. Calcium phosphate treatment was performed during the 24 h before infection (A), during the first 24 h p.i. (B), or during 24 h 2 days p.i. (C). Calcium phosphate precipitates containing HPV-16 DNA (open reading frames E2 to E5 and E6 and E7) did not induce the parvovirus replication cycle as effectively as precipitates free of DNA. Control, Untreated H-1-infected HEF cells 4 days p.i.

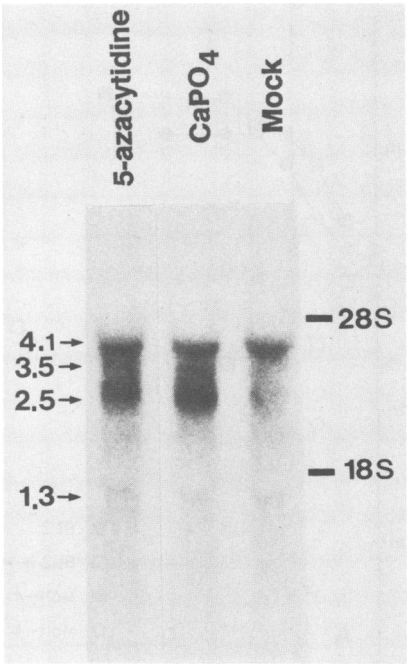

FIG. 3. Cytoplasmic RNA of H-1-infected HEF cells hybridized with ^{32}P-labeled H-1 DNA (21). Untreated HEF cells reveal only 4.1- and 1.2-kb bands. After treatment of the cells with 5-azacytidine or calcium phosphate precipitates, 3.5- and 2.5-kb transcripts were induced. The amounts of RNA in the three lanes were the same.

genes (16). Treatment of the nonpermissive HEF cells with $CaPO_4$ precipitates without DNA and subsequent infection with H-1 virus resulted in activation of parvovirus DNA amplification (Fig. 2) and RNA synthesis (Fig. 3). Similar to the treatment with 5-azacytidine, additional 2.5- and 3.5-kb transcripts were induced by $CaPO_4$ treatment. The H-1 titer of these infected cells measured 4 days p.i. was still below the level of input virus, but six to eight times higher than in untreated cells (Table 2). It could be shown that the input titer measured at 0.5 h p.i. was not modified by treatment with $CaPO_4$. We subsequently analyzed by [^{3}H-*methyl*]thymidine labeling of newly synthesized virions whether the relatively increased H-1 titer was attributable to virus production. If any virus production was induced by calcium phosphate precipitates, this effect clearly was very marginal and not comparable to the effect of calcium phosphate measured for H-1 DNA amplification and RNA expression (data not shown).

In order to analyze whether increased concentration of free intracellular calcium was responsible for the induction of parvovirus RNA expression, the calcium ionophore A23187 was applied to HEF cells 24 h prior to infection. A23187 increases the concentrations of intracellular calcium and was applied in concentrations reported to have biological effects in fibroblasts (8, 17). A23187 had no effect at concentrations between 0.5 and 50 μM on parvovirus RNA expression, and there was no increase in the production of infectious particles.

DISCUSSION

Parvovirus H-1-infected hamsters exhibited a decrease in the incidence of spontaneous or chemically or virally induced tumors compared with uninfected animals (27, 29, 30). Since previous experiments demonstrated that parvovirus H-1 can be propagated in human cells only after cell transformation accompanied by death of the infected cultures (4, 28), it was of interest to investigate whether factors allowing permissive growth of H-1 in human cells are linked to specific functions related to tumorigenesis.

This report confirms that nontransformed diploid human fibroblasts and human keratinocytes are nonpermissive for H-1, i.e., virus particles were not produced nor did CPEs become visible in spite of replication of H-1 DNA. Also, a line of spontaneously immortalized human keratinocytes did not support a full replicative cycle of H-1 virus.

Interestingly, transformation of human fibroblasts by SV40 renders the cells permissive for H-1 (4). Our results with a variety of additional virus-transformed cells indicate that virus-induced transformation provides functions permitting a complete replicative cycle of H-1 in human cells (cytopathic changes and production of infectious particles), irrespective of the type of transforming virus. Similar to primary cells, most transformed cell lines devoid of detectable viral DNA were nonpermissive or only semipermissive for H-1 infection, although they exhibited cytopathic changes following H-1 infection. A recent study demonstrated that cells of breast carcinoma lines not known to contain viral DNA do not produce infectious H-1 particles although they are killed after parvovirus infection (T. Dupressoir, J. M. Vanackes, J. J. Cornelis, N. Duponchel, and J. Rommelaere, Cancer Res., in press). Furthermore, it has been shown that only a small fraction of cells transformed by irradiation or chemicals became permissive for H-1 (5, 6). Thus induction of cytopathogenic changes by parvovirus H-1 appears to be correlated with the transformed phenotype of the infected cells: immortalization (as in HaCaT

cells) seems to be sufficient to induce some cytopathic changes. Whether expression of the CPE-inducing protein of H-1 (NS1) is facilitated or altered by functions present in immortalized or transformed cells remains to be investigated. The data available thus far indicate that in the replicative cycle of H-1 virus, DNA amplification, expression of cytopathic functions, and synthesis of infectious progeny depend on different cellular functions. Our data show that full permissiveness of human cells for H-1 (i.e., production of infectious progeny) is greatly facilitated by or possibly even dependent on the presence of the viral DNA of oncogenic viruses in previously transformed or immortalized cells. However, neither early nor late gene expression of these oncogenic viruses is sufficient to permit propagation of H-1, as shown in this study with SV40 infection.

However, the T-cell leukemia cell line Molt-4 and the Burkitt's lymphoma cell line BJA-B, which until now have not been found to contain viral DNA, were permissive for H-1. Possibly these lines may contain unidentified viral DNA. Since BJA-B was infected with EBV during the early course of cell line establishment and has been passaged in nude mice, it cannot be excluded that this cell line may have been contaminated by other viruses (15). It is possible that these cell lines could contain viruses as yet unidentified.

In nonpermissive primary human fibroblasts, which express only a parvovirus 4.1-kb transcript, additional 2.5- and 3.5-kb transcripts were induced by treatment with 5-azacytidine or calcium phosphate. Both substances have been shown to induce cellular genes (11, 16). This suggests, that permissiveness is regulated by cellular genes not expressed in normal human cells. Since only parvovirus DNA replication and gene expression, but not production of infectious particles, were induced by these substances, additional cellular factors appear to be involved in the complete replicative cycle of H-1.

Hybrids between the permissive HPV-18 DNA-containing cervical carcinoma cell line HeLa and nonpermissive normal human fibroblasts (444 cells) (26) also contained HPV DNA but lost the malignant phenotype. Tumorigenic HeLa, nontumorigenic 444, and tumorigenic CGL3 cells (26), which are malignant revertants of 444, were all permissive for H-1. This seems to emphasize that full permissiveness is linked to virus-induced transformation rather than to the tumorigenic phenotype.

The HPV-18 E6 and E7 open reading frames seem to be under the control of negative regulatory factor(s) acting at the viral 5′ regulatory region (22). One of the enhancer sequences of this region, the ACC(N_6)GGT motif, is also present in the 3′ hairpin of the H-1 genome as a head-to-head dimer. Nearby, four additional ACC(N_4)GGT motifs are found. Another ACC(N_6)GGT motif is found in the regulatory region of the open reading frame coding for the H-1 capsid proteins. Whether these structural similarities are involved in the permissiveness of HeLa cells for H-1 remains to be determined. Indeed there seems to be an interaction at the level of transcriptional control, since in H-1-infected HeLa cells HPV-18 expression is readily inhibited (J. R. Schlehofer, S. Faisst, and H. zur Hausen, manuscript in preparation). This might result from competitive interactions involving the regulatory regions of the genomes.

The data presented here suggest that the tumor-suppressive properties of H-1 infection might result from H-1-induced CPEs in transformed cells. On the basis of the results presented here it is anticipated that the antitumorigenic effect of H-1 should be more pronounced in virus-transformed cells than in other tumor cells since in addition to the CPEs, progeny virus could be provided to infect adjacent cells.

The characterization of the interaction of H-1 with the expression of tumor virus functions, apparently required to maintain the tumorigenic phenotype, may further contribute to the understanding of tumor suppression by parvoviruses.

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LITERATURE CITED

1. **Boukamp, P., R. T. Petrusevska, D. Breitkreutz, J. Hornung, A. Markham, and N. E. Fusenig.** 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell. Biol. **106**:761–771.
2. **Bürkle, A., T. Meyer, H. Hilz, and H. zur Hausen.** 1987. Enhancement of N-methyl-N′-nitro-N-nitrosoguanidine-induced DNA amplification in a simian virus 40-transformed chinese hamster cell line by 3-aminobenzamide. Cancer Res. **47**:3632–3636.
3. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7**:2745–2752.
4. **Chen, Y. Q., F. de Foresta, J. Hertoghs, B. L. Avalosse, J. J. Cornelis, and J. Rommelaere.** 1986. Selective killing of simian virus 40-transformed human fibroblasts by parvovirus H-1. Cancer Res. **46**:3574–3579.
5. **Cornelis, J. J., B. Avalosse, S. Mousset, M. Namba, and J. Rommelaere.** 1986. Selective destruction by parvoviruses of human and mouse fibroblasts transformed with γ-irradiation. Int. J. Radiat. Biol. **49**:529.
6. **Cornelis, J. J., P. Becquart, N. Duponchel, N. Salome, B. L. Avalosse, M. Namba, and J. Rommelaere.** 1988. Transformation of human fibroblasts by ionizing radiation, a chemical carcinogen, or simian virus 40 correlates with an increase in susceptibility to the autonomous parvoviruses H-1 virus and minute virus of mice. J. Virol. **62**:1679–1686.
7. **de Villiers, J., and W. Schaffner.** 1983. Transcriptional enhancers from papovaviruses as components of eucaryotic expression vectors, p. B507/1–B507/20. *In* R. A. Flavell (ed.), Techniques in the life sciences, B5. Nucleic acid biochemistry. Elsevier, Ireland.
8. **Drummond, I. A. S., A. S. Lee, E. Resendez, and R. A. Steinhardt.** 1987. Depletion of intracellular calcium stores by calcium ionophore A23187 induces the genes for glucose-regulated proteins in hamster fibroblasts. J. Biol. Chem. **262**:12801–12805.
9. **Dürst, M., R. T. Dzarlieva-Petrusevska, P. Boukamp, N. E. Fusenig, and L. Gissmann.** 1987. Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. Oncogene **1**:251–256.
10. **Green, M. R., L. Lebowitz, and R. G. Roeder.** 1979. Expression of the autonomous parvovirus H1 genome: evidence for a single transcriptional unit and multiple spliced polyadenylated transcripts. Cell **17**:967–977.
11. **Jones, P. A.** 1985. Altering gene expression with 5-azacytidine. Cell **40**:485–486.
12. **Kerbel, R. S., C. Waghorne, M. S. Man, B. Elliot, and M. L.**

Breitman. 1987. Alteration of the tumorigenic and metastatic properties of neoplastic cells is associated with the process of calcium phosphate-mediated DNA transfection. Proc. Natl. Acad. Sci. USA **84**:1263–1267.
13. **Lebowitz, R. M., and R. G. Roeder.** 1986. Parvovirus H-1 expression: mapping of the abundant cytoplasmic transcripts and identification of promoter sites and overlapping transcription units. J. Virol. **58**:271–280.
14. **Ledinko, N., S. Hopkins, and H. Toolan.** 1969. Relationship between potentiation of H-1 growth by human adenovirus 12 and inhibition of the 'helper' adenovirus by H-1. J. Gen. Virol. **5**:19–31.
15. **Menezes, J., W. Leibold, G. Klein, and G. Clements.** 1975. Establishment and characterization of an Epstein-Barr virus (EBV)-negative lymphoblastoid B cell line (BJA-B) from an exceptional, EBV-genome-negative African Burkitt's lymphoma. Biomedicine **22**:276–284.
16. **Pine, R., D. E. Levy, N. Reich, and J. E. Darnell.** 1988. Transcriptional stimulation by $CaPO_4$-precipitates. Nucleic Acids Res. **16**:1371–1378.
17. **Reed, P. W., and H. A. Lardy.** 1972. A23187: a divalent cation ionophore. J. Biol. Chem. **247**:6970–6977.
18. **Rhode, S. L., III.** 1973. Replication process of the parvovirus H-1 I. Kinetics in a parasynchronous cell system. J. Virol. **11**:856–861.
19. **Rhode, S. L., III.** 1987. Construction of a genetic switch for inducible *trans*-activation of gene expression in eucaryotic cells. J. Virol. **61**:1448–1456.
20. **Rhode, S. L., III, and S. M. Richard.** 1987. Characterization of the *trans*-activation-responsive element of the parvovirus H-1 P38 promoter. J. Virol. **61**:2807–2815.
21. **Rigby, P. W., C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–252.
22. **Rösl, F., M. Dürst, and H. zur Hausen.** 1988. Selective suppression of human papillomavirus transcription in non-tumorigenic cells by 5-azacytidine. EMBO J. **7**:1321–1328.
23. **Schlehofer, J. R., M. Ehrbar, and H. zur Hausen.** 1986. Vaccinia virus, herpes simplex virus and carcinogens induce DNA amplification in a human cell line and support replication of a helper virus dependent parvovirus. Virology **152**:110–117.
24. **Schlehofer, J. R., R. Heilbronn, B. Georg-Fries, and H. zur Hausen.** 1983. Inhibition of initiator-induced SV40 gene amplification in SV40-transformed chinese hamster cells by infection with a defective parvovirus. Int. J. Cancer **32**:591–595.
25. **Shein, J. M., and J. F. Enders.** 1962. Multiplication and cytopathogenicity of simian vacuolating virus 40 in cultures of human tissues. Proc. Soc. Exp. Biol. Med. **109**:495–500.
26. **Srivatsan, E. S., W. F. Benedict, and E. J. Stanbridge.** 1986. Implication of chromosome 11 in the suppression of neoplastic expression in human cell hybrids. Cancer Res. **46**:6174–6179.
27. **Toolan, H.** 1967. Lack of oncogenic effect of the H-viruses for hamsters. Nature (London) **214**:1036–1039.
28. **Toolan, H., and N. Ledinko.** 1965. Growth and cytopathogenicity of H-viruses in human and simian cell cultures. Nature (London) **208**:812–813.
29. **Toolan, H., and N. Ledinko.** 1968. Inhibition by H-1 virus of the incidence of tumors produced by adenovirus 12 in hamsters. Virology **35**:475–478.
30. **Toolan, H., S. L. Rhode, and J. F. Gierthy.** 1982. Inhibition of 7,12-dimethylbenz(a)anthracene-induced tumors in Syrian hamsters by prior infection with H-1 parvovirus. Cancer Res. **42**:2552–2555.
31. **Yakobson, B., T. Koch, and E. Winocour.** 1987. Replication of adeno-associated virus in synchronized cells without the addition of a helper virus. J. Virol. **61**:972–981.
32. **Yalkinoglu, Ö. A., R. Heilbronn, A. Bürkle, J. R. Schlehofer, and H. zur Hausen.** 1988. DNA amplification of adeno-associated virus as a response to cellular genotoxic stress. Cancer Res. **48**:3123–3129.