Association of Adenovirus Type 12 Deoxyribonucleic Acid with Host Cell Chromosomes

HARALD ZUR HAUSEN

Virus Laboratories, The Children's Hospital of Philadelphia, and School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19146

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Preparations of purified ^3H -labeled adenovirus type 12 (^3H -Ad. 12) were analyzed for radioactive impurities by Millipore filtration and ultracentrifugation. It was found that only about 1% of the isotope activity was separable from the virions. Exposure of hamster cell lines to ^3H -Ad. 12 resulted in nonlytic infections, and autoradiography indicated that viral deoxyribonucleic acid (DNA), or parts thereof, became associated with host cell chromosomes. Usually, the label was observed in the form of small clusters of grains, as described previously for lytic adenovirus infections of human embryonic kidney cells. The uptake of labeled virus by the PT-K1 line of rat kangaroo cells was close to background level. These cells did not adsorb the virus to any significant extent. Ultraviolet irradiation of the virus for as long as 8 min did not affect viral adsorption onto susceptible cells, nor did it alter the association of viral DNA with host cell chromosomes. The capacity of the virus to induce chromosomal aberrations decreased linearly with an increase in the dose of irradiation, but the decrease occurred at a rate which was four- to fivefold slower than the rate of inactivation of viral infectivity. These results suggest that the capacity to induce chromosomal aberrations is controlled by viral genes.

The association of adenovirus type 12 deoxyribonucleic acid (DNA) with chromosomes of human embryonic kidney (HEK) cells was reported previously (9). Autoradiography of cells infected with purified ^3H -thymidine-labeled adenovirus type 12 (^3H -Ad. 12) suggested that viral genomes, or parts thereof, usually attach to host cell chromosomes without being incorporated. A small proportion of the viral genomes may become integrated into the host cell chromosomes with continued exposure. The uptake of labeled materials by chromosomes was largely prevented by neutralization of the virus with type-specific antiserum before exposure of HEK cells.

The following report presents evidence that (i) association of viral DNA with host cell chromosomes occurs not only in a lytic infection but is demonstrable in nonlytic adenovirus-hamster cell systems as well; (ii) ultraviolet (UV) irradiation of the virus, within limits, does not prevent adsorption of the virus onto cells nor the attachment of viral DNA to chromosomes; and (iii) the isotope activity of purified ^3H -labeled adenovirus preparations is almost wholly virion-associated.

MATERIALS AND METHODS

Cells. HEK and KB cells were grown as reported previously (9). The NIL (3) and BHK 21 (8) lines of Syrian hamster cells were kept as monolayers in Basal Medium Eagle (BME) containing double-strength amino acids and vitamins and supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$). The PT-K1 line of rat kangaroo cells (7) was kindly furnished by W. W. Nichols, Institute for Medical Research, Camden, N.J. These cells were grown as monolayers in medium RPMI-1629 (BBL) supplemented with 10% FCS and antibiotics. Tube cultures of primary HEK cells were obtained from Flow Laboratories, Rockville, Md.

Virus. The preparation of adenovirus type 12 stock seed and the technique used for infectivity assays were reported elsewhere (9). To label the virus with ^3H -thymidine, the isotope (20 $\mu\text{C}/\text{ml}$; specific activity 6 C/mm) was added for 48 hr during the growth period of the virus in KB cells. Thereafter, the cells were harvested and the virus was purified as described (9), with the exception that ultracentrifugation at $100,000 \times g$ after fluorocarbon treatment was omitted. The fluorocarbon-treated virus suspension was placed immediately on a preformed CsCl gradient. After 3 hr of centrifugation at $73,000 \times g$, the fraction containing the viral band was collected and dialyzed against phosphate-buffered saline (PBS). An equal

number of noninfected cells were treated with the above amount of ^3H -thymidine and were processed in parallel for control purposes. After CsCl-gradient centrifugation, the fraction corresponding to the viral band (based on refractive index) was collected and dialyzed.

Virus adsorption tests. Cells (10^7) were suspended in 3 ml of purified ^3H -Ad. 12 suspension containing 1 mg of "cold" thymidine per ml; the cells were then incubated at 37 C. At various intervals, 0.2-ml volumes were removed, diluted in 0.8 ml of PBS, and centrifuged for 5 min at $1,000 \times g$. The supernatant fluids were assayed for radioactivity in a model 3324 Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

The methods used for immunofluorescence, preparation of metaphases, autoradiography, and UV irradiation of the virus were reported previously (9).

RESULTS

Assay of the purity of the virus preparations. Parts of the ^3H -Ad. 12 and ^3H -thymidine labeled control (^3H -Co) fractions were filtered successively through 100- and 50- μ filters (Millipore Corp., Bedford, Mass.) and were assayed for infectivity and radioactivity before and after filtration. The results (Table 1) revealed a relatively high degree of isotope activity in ^3H -Co; the activity, however, was totally filtrable through 50- μ Millipore filters. In contrast, only about 1% of the radioactivity of the purified viral suspension was filtrable. In agreement with these results, ultracentrifugation at $100,000 \times g$ for 1 hr in a #30 rotor of a preparative centrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) sedimented 97.2% of the radioactivity from the purified viral fraction, whereas all isotope activity remained in the supernatant fluid of ^3H -Co.

Comparison of ^3H -Ad. 12 infection of human, hamster, and ratkangaroo cells. Equal numbers

TABLE 1. Isotope activity of purified ^3H -labeled adenovirus type 12 (^3H -Ad. 12) and control preparations (^3H -Co) before and after Millipore filtration

Prepn	^3H -Ad. 12 ^a			^3H -Co ^b	
	Titer (log ID ₅₀ /ml)	Count/min per ml	Per cent	Count/min	Per cent
Before filtration	6.3	26,712	100	8,971	100
100 μ filtrate	3.5	593	2.2	8,990	100
50 μ filtrate	<1	240	0.9	8,922	100

^a Mean values from three separate experiments.

^b Mean values from two separate experiments.

of HEK, NIL, BHK-21, and PT-K1 cells were exposed in parallel to ^3H -Ad. 12 at an input multiplicity of 1. Metaphase preparations were made 24 hr after infection and were assayed by autoradiography. Counts of labeled chromosomal loci in 100 metaphases (Table 2) revealed that the ratkangaroo cells contained 10 to 20 times less label than the other cultures tested. This difference would have been even greater if single grains, instead of labeled loci, had been counted. Most of the label in PT-K1 cells consisted of single grains, whereas the label usually formed small clusters of grains in the three other types of cells. Since it was often impossible to determine the exact number of grains in clusters, the counts were restricted to labeled loci.

Immunofluorescence tests performed 24 hr after infection revealed that HEK cells exposed to virus at an input multiplicity of 100 contained virus-induced T-antigen, as well as viral capsid antigens. In both hamster cell lines (NIL and BHK-21), only T-antigen production became detectable, and, in the ratkangaroo culture, neither T- nor viral antigens could be demonstrated.

These experiments raised a question: Did the small number of labeled chromosomal loci in the PT-K1 cells and the failure of these cells to produce T-antigen result from insufficient adsorption of the virus, or were these effects caused by the failure of the virus to penetrate or eclipse? To answer this question, KB, NIL, and PT-K1 cells were exposed to ^3H -Ad. 12, and the degree of adsorption at various time intervals was determined by measuring the decrease in isotope activity (5) in the culture fluids (Fig. 1). After 3 hr of adsorption, the isotope activity in the clarified media of PT-K1 cells was not reduced when compared to the activity recorded at the beginning of the experiment. Clearly, the virus was not adsorbed onto PT-K1 cells to any significant extent.

TABLE 2. Labeled loci in 100 metaphases of various cell lines 24 hr after exposure to infection with ^3H -adenovirus type 12 at an input multiplicity of 1

Infected cell line	No. of labeled loci	Percentage of HEK label
HEK ^a	634	100
NIL ^b	405	65.5
BHK ^c	297	47
PT-K1 ^d	31	4.9

^a HEK = human embryonic kidney cells.

^b NIL = Syrian hamster cell line.

^c BHK = Syrian hamster cell line.

^d PT-K1 = ratkangaroo cell line.

FIG. 1. Adsorption of ^3H -labeled adenovirus type 12 onto KB, NIL, and PT-K1 cells as evidenced by decreases in radioactivity of the culture fluid.

Attachment of viral genomes to chromosomes after UV irradiation of the virus. Samples of ^3H -Ad. 12 were exposed to UV irradiation for various periods of time. These preparations, as well as nonirradiated virus, were (i) assayed for infectivity on primary HEK cells; and (ii) used to infect NIL cells at an input multiplicity corresponding to 4. After an adsorption period of 2 hr, the radioactivity in the supernatant fluid of the NIL cultures was determined as a measure of viral adsorption. Metaphase preparations were made 24 hr after infection, and these preparations were examined for chromosomal aberrations and, following autoradiography, for labeled chromosomal loci. As seen in Fig. 2, viral adsorption was not reduced after UV irradiation of the virus for up to 10 min. Likewise, the attachment of viral DNA, or parts thereof, to chromosomes was unaffected when the virus was irradiated for as long as 8 min. With further irradiation, a slight decrease in attachment was seen, and this finding was confirmed in a second experiment. With nonirradiated ^3H -Ad. 12, chromosomal breaks and other chromosomal abnormalities (Fig. 3) were seen in 68% of the metaphases. Labeled loci were usually not associated with the sites of chromosomal aberrations; this finding agrees with previous results obtained in HEK cells (9). After UV-irradiation of the virus, the incidence of chromosomal aberrations decreased linearly as the dose increased, and the label became associated with intact chromosomes (Fig. 4). The infectivity of the virus was inactivated at a four- to fivefold faster rate than its capacity to induce chromosomal aberrations (9).

DISCUSSION

It was shown previously that pretreatment of ^3H -Ad. 12 with type-specific antiserum reduced

the uptake of label by HEK cells at least ninefold. The purity of the labeled viral preparation was further analyzed in the present report. Although all of the infectivity of ^3H -Ad. 12 and about 99% of its radioactivity were retained by 50-m μ Millipore filters, all of the isotope present in uninfected control preparations (^3H -Co) passed through such filters. The relatively high radioactivity of the control materials may be accounted for by the fact that uninfected cells continued to divide in the presence of the isotope, whereas cellular DNA synthesis largely ceased in virus-infected cultures. In agreement with the results of Millipore filtration, it was found that most of the isotope activity of ^3H -Ad. 12 was sedimented by centrifugation at $100,000 \times g$, whereas, in ^3H -Co, isotope activity remained in the supernatant fluid. Thus, it is probable that about 1% of the isotope in ^3H -Ad. 12 represented free ^3H -thymidine. Since the labeled virus was permitted to adsorb onto test cells for 2 hr in the presence of 1 mg of "cold" thymidine per ml, the possibility that the impurities accounted for more than a few grains in the autoradiographic preparations is rather remote. Indeed, the rat kangaroo line did not adsorb ^3H -Ad. 12 to any significant extent. Correspondingly, the number of labeled loci in metaphases of these cells was close to the background level of grains and 10- to 20-fold lower than in hamster or human cells exposed to the same virus concentration. Thus, it may be concluded that nearly all of the intracellular label reflected the presence of viral DNA or parts thereof.

Syrian hamster cells are known to produce adenovirus-induced T-antigen (6), but these cells do not support replication of infectious adeno-

FIG. 2. Effect of UV irradiation on different properties of the virus.

FIG. 3. Localization of grains and chromosomal aberrations in metaphases of NIL cells infected with nonirradiated ^3H -labeled adenovirus type 12. Arrows point to chromosomal alterations: (a) and (b) multiple breaks; (c) "pulverization" of one nucleus and chromosomal rearrangements and breaks in apparently fused cells; and (d) complete destruction of chromosomes with numerous labeled loci.

virus type 12. In spite of the abortive nature of this infection, viral DNA was found to attach to chromosomes as it did in a lytic infection of HEK cells. It is likely that a similar association of viral DNA and chromosomes may exist in other types of adenovirus-invaded cells.

It is noteworthy that results similar to those described were obtained with partially UV-in-

activated ^3H -Ad. 12. Even irradiation for 8 min did not reduce the attachment of viral DNA, or parts thereof, to chromosomes. This finding, combined with the capacity of the virus to induce chromosomal aberrations, may be relevant to the relative resistance of the transforming ability of oncogenic DNA viruses to UV inactivation (4), as well as to the increased oncogenicity of ir-

FIG. 4. Localization of grains in metaphases of NIL cells infected with ^3H -labeled adenovirus type 12 UV-irradiated for 6 min. The label is associated with intact chromosomes. Arrows in (c) point to isochromatid labeling suggestive of integration of viral genomes into host cell chromosomes.

radiated viral preparations in vivo (2). In nonlytic infections by nonirradiated adenovirus type 12, the cells sustained extensive chromosomal lesions which were fatal (Fig. 3) (zur Hausen, *in prepara-*

tion). It is conceivable that the reduced capacity of UV-treated virus to cause chromosomal damage may favor and even enhance other more radiation-resistant functions of the virus.

The results support the view that adenovirus-induced chromosomal changes are probably due to the action of an enzyme controlled by viral genes (9). Extensive UV irradiation of the virus did not affect its adsorption or the association of viral DNA, or parts thereof, with host cell chromosomes; however, chromosomal aberrations decreased linearly as the UV dose was increased, indicative of a single hit event. Thus, it is unlikely that viral capsid proteins or activation of cellular lysosomal enzymes (1) by an indirect action of the virus can account for the chromosomal lesions.

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