Chromosomal Aberrations and Cloning Efficiency in Adenovirus Type 12-infected Hamster Cells

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The fate of hamster cells, abortively infected with adenovirus type 12, has been studied by correlation of chromosomal aberrations with induction of T antigens and cloning efficiency. The incidence of chromosomal changes paralleled to some extent the T antigen formation, but was inversely related to the cloning efficiency of the cells. At an input multiplicity of 100, within 24 hr after infection, nearly all of the cells or metaphases revealed the presence of T antigens and chromosomal lesions, respectively, but no clones of cells were obtained. Inhibition of cellular deoxyribonucleic acid synthesis was not noted during this period. Increasing doses of ultraviolet irradiation reduced, successively, the capacity of the virus to induce chromosomal aberrations and correspondingly improved cloning efficiency of the exposed cells. It is concluded that most, if not all, cells revealing chromosomal lesions 24 hr after infection fail to enter further mitoses.

Adenovirus type 12 induces chromosomal aberrations in human and hamster cells (4, 7, 10, 12) which are referable to the viral genome (12, 13). The virus replicates in human cells and causes their destruction within a short period of time. In hamster cells, replication of viral deoxyribonucleic acid (DNA) is not demonstrable, as a rule, and thus no infectious progeny is produced (3, 6, 11). A small fraction of the infected hamster cells may become transformed (6, 9). These transformed cells synthesize virus-specific T antigens and produce tumors after inoculation into hamsters. Depending upon the viral input multiplicity, a considerable proportion of the hamster cell population may show chromosomal aberrations 24 to 48 hr after infection (2, 12). It was of interest to determine the fate of these altered cells and to provide an answer to the question whether the gross chromosomal abnormalities prevent cellular replication and lead to the death of the cells. As an approach to this question, the cloning efficiency of virus-exposed cells was correlated with the incidence of chromosomal aberrations 24 hr after infection. The results indicate an inverse relation between the frequency of chromosomal lesions and cloning efficiency. Up to a viral input multiplicity of 100, viral capsid proteins do not inhibit hamster cell DNA replication (1).

MATERIALS AND METHODS

Cells. The Nil-2 line of Syrian hamster cells was used in all experiments. The maintenance and origin of these cells has been described (13).

Virus. The origin and preparation of adenovirus type 12 stock virus has been reported in detail (12). The virus preparation used had a titer of $10^{8.3}$ TCID₅₀/ml as determined in primary human embryonic kidney cells (12). Cells were infected in plastic petri dishes (6-cm diameter; Falcon Plastics, Los Angeles, Calif.) containing 2×10^5 cells. For adsorption, the medium was removed and 0.2 ml of virus suspension was added per dish. After 2 hr at 37 C, unadsorbed virus was washed off and fresh medium was added.

Cloning efficiency. The techniques of MacPherson and Montagnier (5) were used. Cells (5×10^4) were seeded in 0.3% agarose (L'Industrie Biologique Francaise S.A., Gennevilliers, France). The cloning efficiency of uninfected Nil-2 cells ranged from 60 to 70%. These control values were taken as 100%. Virus-infected cells were seeded either immediately after the adsorption period or 24 hr later in agarose medium. No significant differences were obtained at these two time intervals. Colonies were scored 7 and again 14 days after seeding under an inverted Leitz microscope. Ten fields each of duplicate petri dishes were counted per given preparation at $\times 35$ magnification.

Autoradiography. At 4 hr after infection, tritiated thymidine (specific activity, 6 c/mm; Schwartz Bio-Research Inc., Orangeburg, N.Y.) was added to the cultures to achieve a final concentration of $0.05 \mu\text{C}/\text{ml}$. Cells were harvested 20 hr later and examined by autoradiography as described (15). For determination of DNA synthesis, 1,000 individual cells were examined per preparation for presence of silver grains.

Methods employed for immunofluorescence, metaphase preparations, and ultraviolet (UV) irradiation of the virus have been recorded (12). Criteria for adenovirus type 12-induced chromosomal aberrations were given previously (12, 13).

RESULTS

Nil cells were infected at different input multiplicities with nonirradiated and UV-irradiated adenovirus type 12. They were examined 24 hr later for presence of T antigens and chromosomal aberrations. At this time, cells were also plated in agarose for determination of their cloning efficiency. The results of a representative experiment (Table 1) show that the capacity of the virus to induce chromosomal aberrations is more resistant to inactivation by UV than its ability to induce T antigens.

Figure 1 demonstrates the dependence of T antigen synthesis, chromosomal aberrations, and cloning efficiency on the input multiplicity of the virus. It is evident that there exists an inverse relation between induction of T antigens and chromosomal aberrations on the one side, and the cloning efficiency on the other. After UV-irradiation for 10 min, the virus reduces the cloning efficiency of the cells only at high input multiplicities and then merely to a minor extent. Under these conditions, a slight increase in chromosomal aberrations was also noted.

TABLE 1. Correlation between metaphases containing chromosomal aberrations, cells with T antigen synthesis, and cloning efficiency in abortively infected cells (Nil-2) before and after irradiation of adenovirus type 12

Virus Treatment	Input multiplicity				
	0	0.1	1	10	100
	%	%	%	%	%
Nonirradiated virus					
T antigen	0	2	29	95	100
Chromosomal aberrations	3 ^a	6	28	86	95
Cloning efficiency	100	100	32	2	0
UV-treated (1 min)					
T antigen	0	<0.1	<0.1	19	96
Chromosomal aberrations		2	16	52	89
Cloning efficiency		100	96	30	0.25
UV-treated (5 min)					
T antigen	0	<0.1	<0.1	<0.1	28
Chromosomal aberrations		5	7	11	38
Cloning efficiency		100	100	68	4
UV-treated (10 min)					
T antigen	0	<0.1	<0.1	<0.1	<0.1
Chromosomal aberrations		3	3	3	11
Cloning efficiency		100	100	100	68

FIG. 1. Relation of adenovirus type 12 input multiplicity on neoantigen synthesis, chromosomal aberrations and cloning efficiency of Nil cells. Symbols: Δ , plating efficiency after infection with nonirradiated virus; $\square$, plating efficiency after infection with inactivated virus (10 min of UV irradiation); $\blacktriangle$, neoantigen-producing cells after infection with nonirradiated virus; $\bullet$, metaphases containing chromosomal aberrations (non-irradiated virus); and $\bullet$, metaphases containing chromosomal aberrations (inactivated virus, 10 min of UV irradiation).

To study the question whether inhibition of cellular DNA synthesis by viral capsid antigens might be responsible for the reduced cellular growth, cells were exposed to ³H-thymidine 4 hr after infection for a total of 20 hr. In contrast to human cells (12), nearly 100% of the hamster cells revealed DNA replication during this period. At input multiplicities of 0, 0.1, 1, 10, and 100, the percentages of labeled nuclei were 97.3, 98.2, 98.4, 98.5, and 99.1, respectively. This indicated that the virus, at the multiplicities used, did not inhibit DNA synthesis in hamster cells.

DISCUSSION

These results demonstrated an inverse relation between cloning efficiency and chromosomal aberrations in adenovirus type 12-infected hamster cells. Induction of T antigens paralleled to some extent the occurrence of chromosomal aberrations, although the first function of the virus was more rapidly inactivated by UV irradiation of the virus than the second. Since transformed cells continue to produce T antigens (3, 8, 11), synthesis of these antigens apparently does not inhibit mitosis. Indeed, early after infection, many of the cells revealed T antigens yet underwent division (14). Chromosomal aberrations due to adenovirus type 12 disappear several

days after infection and no structural abnormalities persist in the surviving cells (10, 12). It seems appropriate, therefore, to ascribe the diminished cloning efficiency to the chromosomal aberrations induced shortly after infection.

The reduction in cloning efficiency exceeded slightly the incidence of chromosomal aberrations. This may indicate that minor chromosomal alterations may occur in additional cells which are microscopically undetected. It is possible, however, that other factors may add to the reduction in cloning efficiency. Since it has been shown that UV-irradiated viral genomes still associate with host cell chromosomes (13), it is conceivable that, at high input multiplicities of infection, this association causes disturbances of DNA replication and subsequent division of the cells.

Cellular DNA replication was not inhibited during the first 24 hr after infection. Since the virus does not replicate in hamster cells, it is unlikely that viral capsid proteins are responsible for the observed inhibition of mitosis.

These various results indicate that induction of chromosomal aberrations ultimately leads to cell death and does not correlate with the oncogenic potential of the inducing agent. It has been shown recently that polyoma virus, a relatively potent "transformer" of several types of cells, produces chromosomal aberrations only rarely, even at high input multiplicities (zur Hausen and Weil, *unpublished data*).

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