microscope studies (*13*) of dispersed nucleoli. After dispersion, the nucleoli formed a "beaded necklace" arrangement, with a 30-Å fiber connecting the beadlike structure. Treatment with deoxyribonuclease broke the fiber whereas ribonuclease, trypsin, and pepsin caused a reduction in size but left the circle intact. The possibility arises that, in the unfertilized egg in which the nuclear membrane has broken down and the chromosomes are in the metaphase of the first maturation division, extruded nucleoli may be significant sources of DNA.

The foregoing results would indicate that isolations of DNA from unfertilized eggs which yield quantities of the order of 0.004 $\mu$g per egg may represent only that DNA which was in the germinal vesicle before meiosis (that is, the chromosomal and nucleolar DNA) and that, at this quantitative level, mitochondria may not be significant sources of egg DNA. The large amounts that have been measured by indirect methods, the so-called cytoplasmic DNA, may also be present but in complexes, localizations, or with properties such that it is not obtainable by conventional methods of preparation.

*Note added in proof*: It has been reported (*14*) that the mitochondrial DNA content of the frog egg is 0.002 $\mu$g. These results and the values reported above for the DNA content of germinal vesicles would suggest that the total DNA content of the fertilized frog egg is at least 0.006 $\mu$g.

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## Replication of Adenovirus Type 7 in Monkey Cells: A New Determinant and Its Transfer to Adenovirus Type 2

Abstract. *A strain of human adenovirus type 7, adapted to replication in green-monkey kidney cells, requires the interaction of two particles to initiate plaque formation in the simian cells. One particle is a true adenovirion. The second, apparently defective, consists of a genome carrying a monkey-adapting component in an adenovirus capsid; this genome does not express known SV40 determinants. The addition of human adenovirus type 7 that is not adapted enhances the titer and changes conditions for plaque formation by the adapted virus to a one-particle requirement. Addition of nonadapted human adenovirus type 2 as helper virus results in the transfer of the monkey-adapting component from adenovirus type 7 to adenovirus type 2. The population containing the adenovirus 2 transcapsidant then has the ability to replicate in simian cells.*

Most strains of human adenovirus are unable to replicate in simian cells unless the cells are coinfected with papovavirus SV40. The necessary growth-potentiating information may be provided by complete, autonomously replicating SV40 (*1*), by complete SV40 genomes encased in adenovirus capsids (*2*), or by defective SV40 (PARA) (particle aiding the replication of adenoviruses). Like a true adenovirion, the PARA particle contains an adenovirus capsid. PARA is unable to replicate in the absence of helper adenovirus, but can be identified by the ability to induce synthesis of SV40 tumor (T) antigen (*3*). Lewis *et al.* (*4*) have reported that many monkey-adapted strains of adenovirus are contaminated with SV40, in that they can induce synthesis of its T antigen.

During an investigation of strains of human adenoviruses able to replicate in simian cells, we encountered a human adenovirus type 7, adapted to growth in monkey cells, which did not induce the synthesis of detectable amounts of SV40 T antigen. The history of this monkey-adapted adenovirus 7, called adeno 7 (M), is as follows. Wyeth Laboratories received a sample of adenovirus type 7 that had been isolated at the National Institutes of Health and had been passed twice in human kidney cells. The virus, designated No. 19690, was then passed once in human embryonic kidney cells, four times in green-monkey kidney cells, and six times in baboon kidney cells. All the cell lots were tested and found free of SV40. The last passage material from the baboon cells, MK10, was negative when tested for PPLO (pleuropneumonia-like organisms) and negative when safety-tested for contaminating viruses in the presence of type 7 antiserum in cells from rabbit kidney, human amnion, and rhesus kidney. Adeno-satellite virus (ASV) particles (*5*) were abundant. We received a portion of the MK10 passage of adeno 7 (M) and passed it twice more in green-monkey kidney cells. Our experiments were performed with the second passage stock, which was free of detectable amounts of ASV particles. Adeno 7 (M) does not induce SV40 T antigen in green-monkey kidney cells detectable by either immunofluorescence or complement fixation.

Adenovirus plaque assays were performed with human embryonic kidney cells in 35-mm plastic petri dishes (*6*). Assays were also performed in green-monkey kidney cells growing in 60-mm plastic petri dishes, sometimes in the presence of high concentrations of nonreplicating helper human adenovirus (*6*). The overlay for all assays contained Eagle's medium, 10 percent fetal bovine serum, 0.23 percent sodium bicarbonate, and 1 percent agar. A second overlay was applied on day 8 and contained neutral red diluted 1:20,000.

Plaque formation by adeno 7 (M) was studied in human embryonic and green-monkey kidney cells with twofold dilutions of the virus. The number of plaques developing in the cultures of human cells closely followed the dilution factor of the virus inoculum, suggesting that infection by only one particle was required to initiate a plaque. This was found to be the case (*6*) with PARA-adenovirus 7, a population known to be carrying defective SV40 genomes.

Plaque formation in green-monkey kidney cells by adeno 7 (M), however, was found to follow second-order kinetics. Five to eight replicate plates were used for 0.1 ml of each twofold dilution of the virus. The results of a representative experiment are shown in Table 1. At a dilution of 1:800, an average of 50 ± 3.9 plaques were counted on each plate. This aver-

Table 1. Plaque formation in green-monkey kidney cells by a monkey-adapted strain of adenovirus type 7 (M) in the presence and absence of helper adenovirus [adenovirus type 7 (H)].

| Dilution | Plaques per plate* | | |
|---|---|---|---|
| | Expected (1 particle) | Expected (2 particles) | Observed |
| *Adenovirus 7 (M)* | | | |
| 1:800 | | | 50 ± 3.9 |
| 1:1600 | 25 | 13 | 17 ± 3.1 |
| 1:3200 | 13 | 3 | 3 ± 0.9 |
| 1:6400 | 6 | 0 | 0 |
| *Adenovirus 7 (M) + helper virus* | | | |
| 1:1600 | | | 65 ± 2.2 |
| 1:3200 | 33 | 16 | 33 ± 4.7 |
| 1:6400 | 16 | 4 | 16 ± 3.5 |
| 1:12800 | 8 | 1 | 6 ± 1.7 |
| 1:25600 | 4 | 0 | 3 ± 1.4 |
| 1:51200 | 2 | 0 | 0.8 ± 0.4 |

* Numbers are averages of 5 to 8 replicate plates.

age dropped to 17 ± 3.1 at a dilution of 1:1600, and another twofold dilution yielded an average of 3 ± 0.9. On statistical analysis the values were found not to differ significantly ($P = .55$) from those expected if the interaction of two particles was required for development of a plaque, but were significantly different ($P = .0003$) from the single-particle requirement.

Addition of large amounts (5 to 10 particles per cell) of nonreplicating human adenovirus type 7, called adeno 7 (H), converted the kinetics of plaque formation by adeno 7 (M) to a one-particle requirement (Table 1). Thirty-three plaques were expected at a dilution of 1 : 3200 if first-order kinetics were operable and an average of 33 ± 4.7 plaques were observed. A twofold dilution yielded 16 ± 3.5 plaques per plate, and another twofold dilution reduced the number of plaques obtained to an average of 6 ± 1.7. A subsequent twofold dilution reduced the number of observed plaques by 50 percent. Chi-square analysis of these results revealed that the observed numbers of plaques were not significantly different ($P = .85$) from the number expected if first-order kinetics were followed in the presence of helper adenovirus, but were now significantly different ($P \leq .0001$) from the two-particle requirement.

The addition of excess amounts of adeno 7 (H) also enhanced, about eightfold, the end-point titer of monkey-adapted adenovirus in green-monkey kidney cells (Table 1). Approximately three plaques were obtained at a dilution of 1 : 3200 in the absence of helper virus. In the presence of non-adapted adeno 7 (H), however, about three plaques were formed at a 1 : 25,600 dilution of adeno 7 (M). These results suggest there is a second particle present in the adeno 7 (M) stock which must interact with an adenovirion in order to initiate plaque formation. Similar patterns of plaque formation had been observed (*6*) with PARA-adenovirus 7, a "hybrid" population containing two types of particles, complete adenovirions and defective SV40 genomes in adenovirus capsids (*3*).

Experiments were designed to determine whether the second particle present in adeno 7 (M) could undergo transcapsidation (*7*). Human adenovirus type 2 was used as helper virus for adeno 7 (M) in green-monkey kidney cells. The titer of adeno 7 (M) was enhanced, similar to the enhancement provided by homotypic adeno 7 (H), (Table 1). Terminal plaques were picked, and the progeny was characterized by neutralization tests in green-monkey kidney cells (Table 2). Plaque formation in these cells in the presence or absence of helper adenovirus type 6 by the parent adeno 7 (M) was not inhibited by antiserum to SV40 but was completely abolished after treatment with rabbit antiserum prepared against a human strain of adenovirus type 7, adeno 7 (H). Similarly, the antiserum to SV40 did not neutralize the progeny from the plaque induced in the presence of helper adenovirus type 2, the progeny being designated adeno 2 (M). However, these progeny were not neutralized by the antiserum to adeno 7 which had inhibited plaque formation by the parent adeno 7 (M). Antiserum against the human strain of adenovirus type 2, adeno 2 (H), which had served as helper virus, neutralized the progeny from the enhanced plaque.

Table 2. Identification of adenovirus 7 (M) and adenovirus 2 (M) by plaque reduction tests with antiserums to adenovirus (H). GMK, green-monkey kidney.

| Inoculum | | Plaques/GMK plate | |
|---|---|---|---|
| Virus | Antiserum to | No helper virus | Helper virus* |
| Adeno 7 (M) | SV40 | 32 | >100 |
| Adeno 7 (M) | Adeno 7 (H) | 0 | 0 |
| Adeno 2 (M) † | SV40 | 3 | 21 |
| Adeno 2 (M) | Adeno 7 (H) | 2 | 14 |
| Adeno 2 (M) | Adeno 2 (H) | 0 | 0 |

* Adenovirus type 6 (H) which replicates in human embryo but not in green-monkey kidney cells. The helper virus is not neutralized by the antiserums to type 7 or type 2 in the inocula. † Progeny from terminal plaque induced by adeno 7 (M) in presence of type 2 helper adenovirus.

Table 3. Ability of adenovirus type 2 to replicate in GMK cells before and after the addition of the monkey-adapting component.

| Virus | PFU per culture | |
|---|---|---|
| | Input | Yield at 72 hours |
| Adenovirus 2 (H) | $3.5 \times 10^5$ | $2.0 \times 10^5$ |
| Adenovirus 2 (M)* | $2.5 \times 10^6$ | $1.0 \times 10^8$ |

* Progeny from terminal plaque induced by adeno 7 (M) in presence of type 2 helper adenovirus; the population now contains the monkey-adapting component.

From the data in Table 2 we also ruled out the possibility that an extraneous agent was involved in plaque formation in green-monkey kidney cells by the adapted virus. Heterologous adenovirus type 6 (5 to 10 particles per green-monkey kidney cell) was used as helper virus. This procedure increased average plaque counts by non-neutralized adeno 7 (M) from 32 to more than 100 and enhanced the average plaque count of nonneutralized adeno 2 (M) from 3 to about 20. Neither adenovirus (M) population treated with the homologous antiserum induced the formation of any plaques in the presence of adeno 6 (H) helper virus. This showed that the monkey-adapting component which interacts with helper adenovirus to cause plaque formation in green-monkey kidney cells is encased in an adenovirus capsid, for it is also neutralized by specific adenovirus antiserum. Otherwise, plaques would have formed when excess nonneutralized helper virus was supplied. Thus, these results demonstrated that transcapsidation had occurred and that the determinant or determinants of the monkey-adapting component had become encased in an adenovirus 2 capsid.

The progeny derived from the plaque enhanced by adenovirus 2 were able to replicate in green-monkey kidney cells (Table 3). Human adenovirus type 2 used as the helper virus initially did not increase in titer during the 72-hour observation period; $3.5 \times 10^5$ plaque-forming units (PFU) were inoculated and $2.0 \times 10^5$ PFU were recovered from the culture. The progeny from an enhanced plaque, now carrying monkey-adapting component, could replicate in the green-monkey kidney cells. The titer of adeno 2 (M) increased from an inoculum of $2.5 \times 10^6$ PFU

to 1.0 × $10^8$ PFU per culture 72 hours later. The type 2 adenovirus had acquired the ability to replicate in green-monkey kidney cells after interaction in these cells with the monkey-adapting component present in the adeno 7 (M) stock. This growth capability was also demonstrated (Table 2) by the fact that plaques formed in green-monkey kidney cells after inoculation of adeno 2 (M). Growth analyses in monkey cells (*8*) have revealed that the monkey-adapting component does not replicate in the absence of helper adenovirus, a situation similar to that of the PARA (defective SV40) component carried by other adenovirus populations (*6*).

Thus plaque formation in green-monkey kidney cells by a monkey-adapted strain of adenovirus 7 requires the interaction of two particles, both of which are neutralized by adenovirus antiserum. Addition of nonadapted adenovirus type 2 as the helper adenovirus results in adenovirus 2 progeny, which carry the determinant or determinants of the monkey-adapting component and are able to replicate in green-monkey kidney cells. These results demonstrate that not every monkey-adapted adenovirus carries information which codes for the synthesis of SV40 T antigen, for the adenovirus populations carrying the monkey-adapting component do not induce SV40 T antigen. However, the monkey-adapting component adenovirus being studied may be carrying SV40 genetic information defective not only for viral structural determinants, but also for the tumor antigen marker.

Currently the origin of the information in the monkey-adapting component is unknown. The monkey-adapting component is the second example of a foreign determinant being carried by adenoviruses, the first example being known SV40 determinants. In both instances, acquisition of the foreign determinants enables the adenovirus to replicate in green-monkey kidney cells.

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## Liver Cancer: Neonatal Estrogen Enhances Induction by a Carcinogen

Abstract. *A single injection of 100 micrograms of estradiol benzoate into newborn rats was followed after weaning by dietary treatment with one of two dosages of the carcinogen N-hydroxy-N-2-fluorenylacetamide. Autopsies 26 weeks later showed a higher incidence of liver cancer in male and, particularly, female rats injected with hormone than in controls. The weights of livers were greater but gonads were smaller in size in the estradiol groups. Endocrine and possibly central-nervous-system factors may play roles in formation of liver tumors.*

Injection of hormones into infant rats has remarkable effects, expressed later in the adults by altered behavioral and physiological responses. The mechanisms involved, especially in regard to sexual activity, are apparently mediated by the central nervous system (*1*).

This technique has now been used to determine the role of hormones and underlying pituitary-hypothalamic controlling elements in the carcinogenic process—for the study of tumor formation in the liver (*2*). The liver of male rats is generally more susceptible to carcinogenic aromatic amine derivatives such as *N*-2-fluorenylacetamide and its active metabolite *N*-hydroxy-*N*-2-fluorenylacetamide (*3*), so induction of liver cancer was studied in male and female rats given a single dose of estrogen at birth.

Rats of the Fischer strain were injected subcutaneously within 24 hours of birth with 100 μg of estradiol benzoate suspended in 0.03 ml of a 1-

Table 1. Effect of a single neonatal injection of estradiol benzoate on physiological parameters and on formation of liver tumors. N-OH-FAA, *N*-hydroxy-*N*-2-fluorenylacetamide; upon weaning at 4 weeks of age the groups on 80 ppm received the carcinogenic diet for 20 weeks and then control diet for 6 weeks; groups on 160 ppm carcinogen were so fed for 16 weeks and then placed on control diets for 10 weeks. Lesions in livers were graded by the system of Reuber (*6*). Female rats in groups receiving 80 ppm N-OH-FAA and no hormone had more foci of hyperplasia and fewer of the more advanced hyperplastic nodules than the groups on 80 ppm treated with estrogen.

| Conc. N-OH-FAA (ppm) | Estradiol | Rats (No.) | Weight* | | | Liver (rats, No.) | |
|---|---|---|---|---|---|---|---|
| | | | Body (g) | Liver (g/100 g) | Gonad (g), ovary (mg) | Hyperplasia | Cancer |
| | | | *Males* | | | | |
| 0 | Yes | 10 | 284 ± 8 | 2.54 ± 0.10 | 1.54 ± 0.25 | 0 | 0 |
| 80 | Yes | 10 | 249 ± 9 | 5.82 ± .40† | 1.47 ± .19 | 2 | 8 |
| 80 | No | 10 | 254 ± 6 | 4.76 ± .26† | 2.54 ± .07 | 5 | 5 |
| 160 | Yes | 15 | 236 ± 4 | 7.81 ± .44 | 1.51 ± .19 | 2 | 13 |
| 160 | No | 11 | 248 ± 4 | 7.46 ± .41 | 2.76 ± .03 | 1 | 10 |
| | | | *Females* | | | | |
| 0 | Yes | 12 | 211 ± 5 | 2.51 ± 0.06 | 26.2 ± 3.2 | 0 | 0 |
| 80 | Yes | 13 | 206 ± 3 | 3.87 ± .14† | 23.1 ± 1.8 | 13 | 0 |
| 80 | No | 8 | 178 ± 2 | 2.84 ± .07† | 51.6 ± 2.5 | 8 | 0 |
| 160 | Yes | 22 | 185 ± 2 | 5.30 ± .20† | 24.9 ± 1.6 | 13 | 9 |
| 160 | No | 16 | 173 ± 3 | 3.58 ± .10† | 54.0 ± 1.6 | 16 | 0 |

* Mean and S.E.; the larger standard error in the liver weights in some groups reflects the more or less extensive and advanced development of lesions. † $P < .05$, by Student's *t*-test, between appropriate groups shown (estradiol "yes" or "no" at each dosage).