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DNA methylation in adenovirus, adenovirus-transformed cells, and host cells

(transformation/thin-layer chromatography of methylated bases)

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ABSTRACT **DNAs of adenovirus type 2 and type 12 contain low amounts of methylated bases (0.01 and 0.02% N^6-methyladenine per adenine, if any, and 0.04 and 0.06% 5-methylcytosine per cytosine for type 2 and type 12, respectively), whereas the DNA of the mammalian host cells contains much more 5-methylcytosine (3.57% for human KB cells). The DNA of hamster cells transformed by adenovirus type 12 contains 3.11 and 3.14% 5-methylcytosine (HA12/7 and T637 cells, respectively), whereas the DNA from untransformed hamster cells (BHK21 cells) contains 2.22% 5-methylcytosine. In the DNA of human and hamster cells, little, if any, N^6-methyladenine was detected.**

Methylation of DNA was determined by a sensitive method based on two consecutive steps of two-dimensional thin-layer chromatography of the radioactively labeled DNA bases. By this procedure the detection limits of 5-methylcytosine and N^6-methyladenine could be lowered to 0.01% per main base.

During replication of adenoviruses in permissive human cells, a large amount of viral DNA is synthesized in the nucleus of the host cell (1–3). Viral DNA accounts for 30–50% of the total intracellular DNA at late times after infection (2, 3). Concomitantly with the onset of viral DNA replication, host DNA synthesis is drastically reduced (4).

In previous DNA base analyses of mammalian cells 3–6% 5-methylcytosine (MeCyt) per cytosine (Cyt) was found (5, 6), while N^6-methyladenine (MeAde) could not be detected (7, 8).

Little is known about the biological role of DNA methylation. There are reports on tissue specificities in the content of MeCyt in mammalian cells (5, 6, 9). Certain developmental stages are associated with variations in the content of methylated DNA bases in pro- and eukaryotes (10, 11). The only established function of certain DNA methyltransferases is the modification of DNA in bacteria leading to resistance against restriction endonucleases of the same specificity (12, 13).

Since it is presumed that synthesis of adenovirus DNA in human cells is mediated, at least in part, by the host replication system, one would expect viral DNA to show the methylation pattern characteristic of host DNA. However, in contrast to host DNA, adenovirus DNA has a low level of methylation. Comparison of untransformed cells and cells transformed by adenovirus has shown a difference in the levels of DNA methylation.

MATERIALS AND METHODS

Cells and Virus. Human KB cells (14) were obtained from the American Type Culture Collection. Baby hamster kidney (BHK21) cells (15) were a gift of Dr. Faulkner, Hamilton, Ontario, Canada. T637 cells (16) are BHK21 cells transformed by adenovirus type 12 (Ad12); the HA12/7 cell line was derived from primary Syrian hamster kidney cells by transformation with Ad12 (17). Human embryonic kidney (HEK) cells were purchased from Flow Laboratories and were passaged two to four times.

Propagation of Cells. KB and HEK cells were grown in Eagle's medium (18) supplemented with 10% calf serum (Gibco). The BHK21, T637, and HA12/7 cell lines were propagated in reinforced Eagle's medium (19) enriched with 10% fetal calf serum (Flow Laboratories).

Propagation and Purification of Virus. KB cells growing in suspension culture were infected with CsCl-purified preparations of adenovirus type 2 (Ad2) or Ad12 at multiplicities of infection of 20 plaque-forming units per cell as described (20). Virus purification has been described elsewhere (3).

Radioactive Labeling of Cellular and Viral DNA. [2-^{3}H]Adenosine (specific activity 21 Ci/mmol) and [6-^{3}H]uridine (specific activity 10 Ci/mmol) were purchased from Amersham-Buchler, Brunswick, Germany. Cells growing in monolayers were incubated at 37° in full medium in the presence of 2.5 μCi/ml each of [2-^{3}H]adenosine and [6-^{3}H]uridine for 2–3 days.

Ad2 or Ad12 DNA was labeled by adding 1 μCi/ml of [2-^{3}H]adenosine and 1 μCi/ml of [6-^{3}H]uridine to adenovirus-infected KB cells in suspension culture 2 hr after infection. The infected cells were harvested and the virus was purified 36–40 hr after infection. In some experiments the ^{3}H-labeled compounds were added 24 hr after infection.

Preparation of Viral and Cellular DNA. Viral DNA was extracted from CsCl-purified virus by the sodium dodecyl sulfate–Pronase B–phenol method described elsewhere (21).

For extraction of cellular DNA, cells growing in monolayers were washed five times each with 5 ml of phosphate-buffered saline deficient in Ca^{2+} and Mg^{2+} (22). The cells on each plate were lysed with 1 ml of 0.5% sodium dodecyl sulfate, 0.1 M Tris·HCl (pH 7.5), 0.02 M EDTA containing 500 μg of Pronase B (Calbiochem). The method of further purification has been described (21).

In some experiments the total cellular DNA and the nonencapsidated viral DNA were isolated from Ad2-infected KB cells 40–48 hr after infection. Cell extracts were prepared by ultrasonic treatment, and the virions were isolated by equilibrium sedimentation in CsCl density gradients with a mean density of 1.334 g/cm^3 as described (3). All free intracellular DNA was recovered from the pellet of this gradient and recentrifuged to equilibrium in a CsCl density gradient with a mean density of 1.70 g/cm^3.

Purification of Viral and Cellular DNA. The DNA was precipitated with two volumes of ethanol and kept for 4 hr at −20°. Traces of RNA were removed by hydrolysis in 0.25 M KOH 16–18 hr at 37°. Subsequently, the DNA was again pre-

Abbreviations: Ade, adenine; Cyt, cytosine; Thy, thymine; Gua, guanine; MeCyt, 5-methylcytosine; MeAde, N^6-methyladenine; Ad2 and Ad12, adenovirus type 2 and type 12, respectively.

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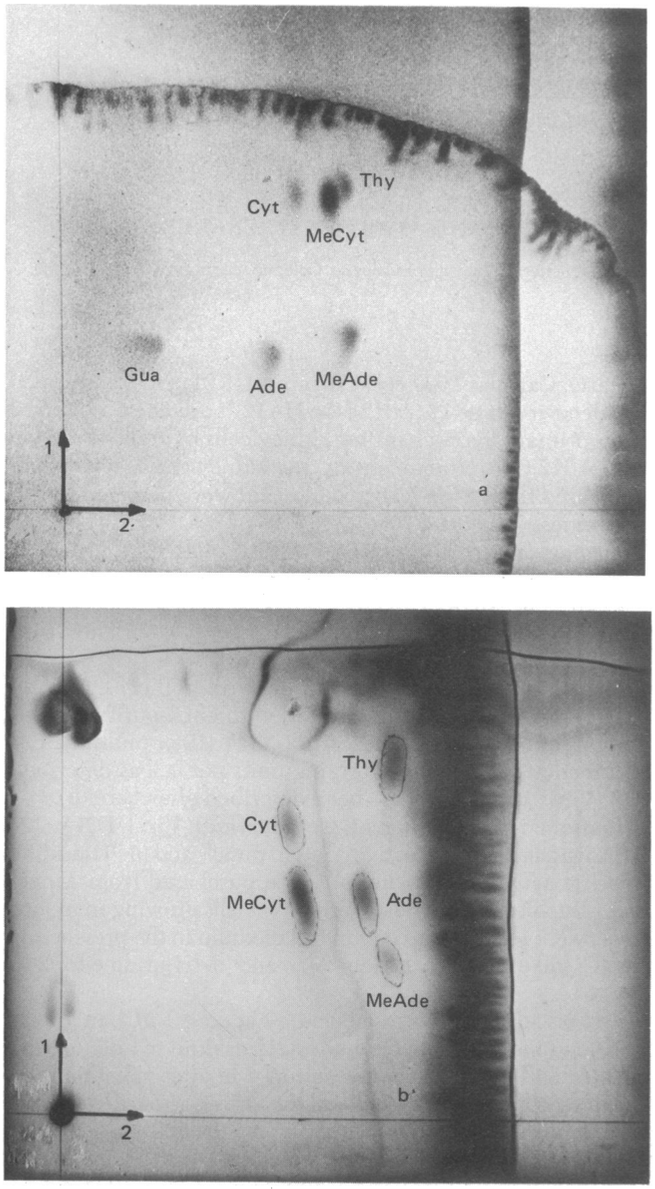

FIG. 1. Thin-layer chromatography of a hydrolysate of Ad12 DNA. (a) Two-dimensional thin-layer chromatography on cellulose of a hydrolysate of Ad12 DNA. Solvents 1 and 2 were used in dimensions 1 and 2, respectively. MeAde and MeCyt (1 μg each) were added as markers. Gua, guanine; Ade, adenine; Cyt, cytosine; Thy, thymine. (b) The MeCyt and thymine spots from the experiment shown in (a) were rechromatographed. Cyt, Ade, and MeAde (1 μg each) were added as markers.

cipitated by adding two volumes of ethanol. The DNA precipitate was washed three to four times with 70% ethanol to remove residual RNA bases.

DNA Hydrolysis. The DNA precipitate and 5 μg each of MeCyt and MeAde as markers in 1.5 ml of formic acid (90%) were sealed in glass tubes and incubated for 30 min at 175° (23). After the tubes were slowly cooled to −70°, they were opened and the hydrolysates were evaporated to dryness. The DNA bases were redissolved in 100 μl of H_2O. Subsequently, 5-μl samples were used to measure base content and radioactivity. The specific activities of the hamster cell DNAs were 5 to 8 × 10^4 cpm/μg of base; those of the human and the viral DNAs, 4 × 10^4 cpm/μg of base.

Base Analysis. Between 10 and 20 μg of hydrolyzed bases were spotted on cellulose thin-layer glass plates (Cel 300-10 UV_{254}, Macherey and Nagel, Düren, Germany) and developed in two dimensions with solvents 1 (water) in dimension 1, and 2 (*n*-butanol:methanol:H_2O:NH_4OH, 60:20:20:1) in dimension 2, (24, 25) (Fig. 1a). MeCyt and thymine (Thy) chromatograph closely together but are well separated upon rechromatography. Adenine (Ade), MeAde, cytosine (Cyt), MeCyt (+ Thy) were marked under a UV lamp, and the spots were eluted with 1.5 ml of 0.1 M HCl. The eluates were evaporated at 70° and redissolved in 50 μl of 0.1 M HCl. They were then spotted on silica gel plastic sheets (Sil NHR UV_{254}, Macherey and Nagel) together with the unlabeled marker bases as controls and rechromatographed in two dimensions with solvents 1 and 2 (Fig. 1b for MeCyt/Thy). After rechromatography, the spot for each base was cut out; radioactivity was determined in Bray's scintillation fluid. Chemoluminescence of the plastic sheet fragments was prevented by keeping the samples for 24 hr in the dark at 4° before radioactivity was determined.

Table 1. Incorporation of [2-^{3}H]adenosine and [6-^{3}H]uridine label into DNA bases

DNA from	[2-^{3}H]Adenosine label*		[6-^{3}H]Uridine label†	
	% of label in Ade	% of label in Gua	% of label in Cyt	% of label in Thy
BHK21 cells	99.5	0.5	36	64
T637 cells	99.6	0.4	38	62
HA12/7 cells	99.6	0.4	42	58
KB cells	99.5	0.5	39	61
HEK cells	99.6	0.4	35	65
Ad2	99.7	0.3	54	46
Ad12	99.7	0.3	50	50
Ad2-infected KB cells				
Labeled 2–12 hr after infection	99.3	0.7	34	66
Labeled 24–48 hr after infection	99.1	0.9	47	53

* Ade + Gua = 100%.
† Cyt + Thy = 100%.

Preparation of Labeled Marker Methylated Bases, [^{3}H]MeCyt and [^{14}C]MeAde. [^{3}H]MeCyt was prepared starting from 5-hydroxymethylcytosine (26). [^{14}C]MeAde was prepared by reacting 6-chloropurine with [^{14}C]methylamine (27).

RESULTS

For determination of small amounts of methylated bases, a reproducible, quantitative method is needed. Two consecutive steps of two-dimensional thin-layer chromatography fulfill these requirements. The rationale for labeling cytosine and adenine is that after incorporation into the growing DNA strands these bases are methylated to a certain extent by specific DNA methyltransferases. Thus, the specific radioactivity of Ade equals that of MeAde, and the specific radioactivity of Cyt that of MeCyt. After isolating these bases and determining the percentage of MeAde/Ade and MeCyt/Cyt, one can calculate the number of methylated bases per DNA genome.

Labeling of DNA. The radioactive compounds used to label DNA were [2-^{3}H]adenosine and [6-^{3}H]uridine. In adenosine ^{3}H label in the 2 position of the purine structure was preferred over that in the 8 position, since almost all of the ^{3}H label from the 2 position appeared in adenine (Table 1). In contrast, after

Table 2. Separation of DNA bases on thin-layer chromatograms*

	Labeled reference substance used			
	[^{3}H]Ade	[^{14}C]MeAde	[^{14}C]Cyt	[^{3}H]MeCyt
	(a) Cellulose thin-layer chromatography			
Ade	*100*	0.6	<0.1	<0.1
MeAde	0.5	*100*	<0.1	0.1
Cyt	0.2	<0.1	*100*	1.6
MeCyt	<0.1	0.1	1.7	*100*
Thy	<0.1	0.1	0.2	2.3
	(b) Silica gel thin-layer chromatography			
Ade	*100*	0.5	<0.1	0.2
MeAde	1.8	*100*	<0.1	0.2
Cyt	0.1	<0.1	*100*	0.5
MeCyt	1.2	0.4	0.7	*100*
Thy	<0.1	<0.1	<0.1	<0.1
	(c) Combined system (cellulose thin-layer chromatography followed by rechromatography on silica gel)			
Ade	*100*	0.01	ND†	ND
MeAde	0.01	*100*	ND	ND
Cyt	ND	ND	*100*	0.01
MeCyt	ND	ND	0.01	*100*
Thy	ND	ND	ND	ND

* Mixtures of the bases Ade, MeAde, Cyt, MeCyt, and Thy and one of the labeled bases [^{3}H]Ade, [^{14}C]Cyt, or [^{3}H]MeCyt were separated by thin-layer chromatography as indicated, and the overlap of the labeled base into the areas of each of the reference substances was determined. Values given are % of label recovered.

† Not detectable.

labeling with [8-^{3}H]adenosine, about 80% of the label in DNA was metabolized to deoxyguanosine (data not shown). Since cytidine compounds, which are tritiated in the 6 position of the pyrimidine ring, were not available, we used [6-^{3}H]uridine, which is readily converted to dCTP and dTTP. Tritium in the 5 position of pyrimidines is not only chemically unstable (at low pH hydrogen exchange occurs), but also biologically unsuitable, since the label is removed during the methyl-group transfer. Most of the radioactivity in the [5,6-^{3}H]cytidine is located in the 5 position and not distributed equally between the 5 and 6 positions (28). However, only the ^{3}H label in the 6 position is chemically and biologically stable in pyrimidines (29).

Thin-Layer Chromatography. The four main bases and the two methylated bases of DNA are well separated on cellulose or on silica gel thin layers using solvents 1 and 2 for either two-dimensional system (Fig. 1a and b). Although Cyt and MeCyt and Ade and MeAde appear optically well separated, the radioactivity from each of these spots spreads into neighboring areas (Table 2a and b). The overlap of the adenine radioactivity into the MeAde spot amounts to 0.5% on cellulose thin-layer chromatography and 1.8% on silica gel thin-layer chromatography. The cytosine radioactivity overlaps into the MeCyt region up to 1.7% on cellulose and 0.7% on silica gel thin-layer chromatography. These values are far too high for analysis of methylated bases, since the lower limit to be tolerable is 0.02%. To reduce overlap from the main bases to the methylated bases, two consecutive two-dimensional thin-layer chromatography systems are necessary. After separation on cellulose thin-layer chromatography, the relevant bases were therefore eluted and each base was rechromatographed individually on silica gel thin-layer chromatography. By this technique, the overlap can be reduced to 0.01% of the adenine and cytosine radioactivities into the areas of the corresponding methylated bases. Thus, the detection limit of MeAde and MeCyt is 0.01% per main base (Table 2c). Moreover, the method shows good reproducibility. The data presented in Table 3 document that the percentage values of MeCyt and MeAde do not vary by more than 10% for one type of DNA. Thus, this coupled two-dimensional thin-layer chromatography system permits the determination of both methylated bases in DNA in a single operation.

Deamination. Acid hydrolysis leads to some deamination of cytosine to uracil, of MeCyt to thymine, and of adenine to hypoxanthine, but does not affect MeAde. The extent of deamination under the experimental conditions used here was determined with radioactively labeled bases as standards. Upon formic acid hydrolysis, about 1% of adenine, cytosine, and MeCyt are converted to the respective deamination products. When, in the next step of the analysis, the bases are eluted with HCl and evaporated at temperatures not exceeding 70°, cytosine and MeCyt are deaminated by 4–5%, and adenine by 1%. Since cytosine and MeCyt are deaminated to the same degree, deamination is irrelevant for the calculation of MeCyt/Cyt. The minor deamination of adenine was neglected, since the deviation is below the detection limits with these low amounts of MeAde.

Methylation of DNA in Mammalian Cells. Several cell lines were analyzed for the extent of DNA methylation (Table 3). In all the cell lines or strains investigated little or no MeAde was detected; the values of MeAde ranged from 0.01% in T637 cells to 0.02% in KB and HEK cells. This finding confirms previous reports in which the absence of MeAde was noted in eukaryotic cells (7, 8). On the other hand, the DNA of all cell lines examined contained relatively high amounts of MeCyt. The MeCyt content of DNA from BHK21 cells was 2.22%. This value was in fairly good agreement with that reported elsewhere (30). The MeCyt levels in the DNA from hamster cells transformed by Ad12 were significantly (about 40%) higher than in nontransformed BHK21 cells. This holds true for the T637 line and the HA12/7 line; the MeCyt levels were 3.11% and 3.14%, respectively (Table 3). The DNA of the human KB line contained 3.57% MeCyt. The level of MeCyt in primary human embry-

Table 3. Methylated bases in mammalian cell DNA and viral DNA

Source of DNA	Exp. no.	Ade (cpm)*	MeAde (cpm)*	MeAde/Ade (%)	Mean	Cyt (cpm)*	MeCyt (cpm)*	MeCyt/Cyt (%)	Mean
BHK 21	1	96.060	12	0.012		57.270	1.287	2.19	
	2	90.546†	12	0.013		90.812	2.045	2.20	
		93.427	14	0.015	0.01	88.984	2.021	2.22	2.22
	3	99.493	25	0.025		96.869	2.185	2.21	
	4	318.451	38	0.012		226.623	5.330	2.30	
T 637	1	144.436	12	0.008		83.709	2.622	3.03	
	2	224.643	15	0.007		100.062	3.277	3.17	
		249.671	21	0.008	0.01	113.712	3.489	2.98	3.11
	3	253.962	15	0.006		101.068	3.370	3.22	
	4	204.247	24	0.012		133.501	4.333	3.15	
HA 12/7	1	390.775	54	0.014	0.02	161.259	5.289	3.18	3.14
		459.398	71	0.016		181.541	5.789	3.10	
HEK	1	25.952	8	0.030		21.311	1.008	4.51	
	2	103.723	15	0.014	0.02	73.840	3.258	4.22	4.40
		117.291	13	0.011		81.212	3.806	4.46	
KB	1	48.268	20	0.041		28.162	1.065	3.64	
	2	124.695	25	0.020		164.333	6.155	3.61	
	3	167.828	19	0.011	0.02	170.851	6.183	3.50	3.57
		166.547	24	0.014		178.340	6.509	3.52	
	4	171.343	45	0.026		137.071	5.125	3.60	
Ad2-infected KB cells Labeled 2–12 hr after infection	1	133.370	9	0.008	0.01	62.454	2.273	3.64	3.53
		130.398	8	0.006		70.473	2.487	3.41	
Labeled 24–48 hr after infection	1	132.627	15	0.011	0.01	53.863	194	0.36	0.35
		131.474	14	0.011		54.862	193	0.35	
Ad2	1	218.798	11	0.005		321.638	40	0.012	
		230.631	3	0.001		329.892	111	0.033	
	2	51.903	19	0.036	0.01	70.749	45	0.063	0.04
		51.570	9	0.017		67.257	22	0.033	
	3	62.555	7	0.011		26.272	11	0.040	
Ad12	1	45.344	7	0.015	0.02	30.483	19	0.062	0.06
		39.425	12	0.030		28.088	19	0.068	

* All values have been corrected for a background of 20 cpm.
† The brackets designate different thin-layer chromatography analyses performed on the same DNA preparations.

onic kidney (HEK) cells was markedly higher (4.40%, Table 3).

It will be of interest to investigate whether the increased extent of methylation in hamster cells transformed by Ad12 is due to a direct enzymatic process—e.g., an increased activity of DNA methyltransferases—or to an indirect effect on the activity of the transferase system as a consequence of the membrane alteration in virus-transformed cells. It has been shown that in the cell lines studied here that had been transformed by Ad12, only a few viral genes are expressed (31). It can be ruled out that the increased MeCyt levels are due to altered precursor uptake, since methylation takes place after DNA synthesis.

In comparison to nontransformed hamster cells, elevated levels of MeCyt have been reported for cells transformed by polyoma virus (30) and Rous sarcoma virus (32).

Undermethylation of Adenovirus DNA. Although the DNA of the host cells in which adenoviruses are propagated (the human KB cell line) has a high level of MeCyt (3.57%), the DNAs of Ad2 and Ad12 contain only 0.04% and 0.06% of MeCyt and 0.01% and 0.02% of MeAde, respectively (Table 3). Note that the limit of detection for methylated bases is about 0.01%. Thus, 0.02% of MeAde is well above background. From these data we estimate that approximately 2 to 3 MeAde and 8 to 9 MeCyt bases per Ad2 and Ad12 genome are present (Ad2 DNA: 2.2×10^7 daltons, 55% G+C; Ad12 DNA: 2.0×10^7 daltons, 49% G+C). Similarly low levels or absence of DNA methylation has been reported for polyoma virus DNA (33). The lack of methylation in adenovirus DNA can be explained by the absence of specific nucleotide sequences that are essential for recognition and the subsequent methylation by the DNA methyltransferases. Another possibility is the induction of an inhibitor of the methyltransferase system at the time of viral DNA replication late in adenovirus infection.

The MeCyt content in the nonvirion DNA, i.e., in the cellular and the nonencapsidated, free viral DNA, was measured in cells infected with Ad2 early (2–12 hr after infection) and late (24–48 hr after infection) in the infection cycle. At an early stage of infection, the MeCyt content of the host DNA is the same as in uninfected KB cells, whereas at late times after infection, methylation levels decrease markedly (0.35% MeCyt/Cyt) (Table 3). At late times after infection, only a decreased level

of cellular DNA synthesis can be detected (10–30%) (4). Thus, the drop in methyl content parallels the decreased amount of cellular DNA synthesized.

DISCUSSION

Although adenovirus DNA and host DNA are both replicated in the nucleus of the cell, the MeCyt content of cellular DNA is higher than that of adenovirus DNA by a factor of 50 to 100 times. In hamster cells transformed by Ad12, the MeCyt levels are approximately 40% higher than in the BHK21 cell line that had not been transformed by virus. The significant increase in the extent of DNA methylation in transformed hamster cells could be due either to a virus-induced methyltransferase or to an enhancement of the activity of host enzymes.

An explanation for the low methylation of adenovirus DNA could be a lack of recognition sites for host methyltransferases. This explanation appears unlikely, since a DNA the size of adenovirus DNA should have methyl group acceptors. Another possibility could be the predominant association of the methyltransferase system with the chromatin structure in the host cell. A third explanation for the low methylation of adenovirus DNA in human KB cells was suggested by experiments with bacteriophage T7. T7 DNA, in contrast to most other DNAs from bacterial viruses, is very poorly methylated *in vivo* (11). However, T7 DNA accepts methyl groups *in vitro* (34). The virus T7 overcomes the restriction barrier of the cell by a drastic alteration of the permeability of the cell membrane (35). The subsequent decrease in ionic strength in the cell (K. H. Altendorf, H. Ponta, M. Schweiger, M. Hirsch-Kauffmann, and M. Pfennig-Yeh, in preparation) appears to inactivate host enzymes, for instance, restriction nucleases and possibly DNA methyltransferases. Thus, the low ionic strength paralyzes the host cell. On the other hand, the newly synthesized T7 viral enzymes are active at low ionic strengths (36). The undermethylation of adenovirus DNA suggests that this virus may use basic mechanisms to overcome the host cell similar to those of virus T7.

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1. Ginsberg, H. S., Bello, L. J. & Levine, A. J. (1967) in *The Molecular Biology of Viruses*, eds. Colter, J. S. & Paranchych, W. (Academic Press, New York), pp. 547–572.
2. Piña, M. & Green, M. (1969) *Virology* **38**, 573–586.
3. Doerfler, W. (1969) *Virology* **38**, 587–606.
4. Burger H. & Doerfler, W. (1974) *J. Virol.* **13**, 975–992.
5. Kappler, J. W. (1971) *J. Cell. Physiol.* **78**, 33–36.
6. Vanyushin, B. F., Mazin, A. L., Vasilyev, V. K. & Belozersky, A. N. (1973) *Biochim. Biophys. Acta* **299**, 397–403.
7. Lawley, P. D., Crathorn, A. R., Shah, S. A. & Smith, B. A. (1972) *Biochem. J.* **128**, 133–138.
8. Craddock, V. M. (1970) *Biochim. Biophys. Acta* **240**, 376–383.
9. Vanyushin, B. F., Tkacheva, S. G. & Belozersky, A. N. (1970) *Nature* **225**, 948–949.
10. Adams, R. L. P. (1973) *Nature New Biol.* **244**, 27–29.
11. Günthert, U. (1975) Dissertation, Dr. rer. nat., Universität Tübingen.
12. Klein, A. & Sauerbier, W. (1965) *Biochem. Biophys. Res. Commun.* **18**, 440–445.
13. Günthert, U., Stutz, J. & Klotz, G. (1975) *Mol. Gen. Genet.* **142**, 185–191.
14. Eagle, H. (1955) *Proc. Soc. Exp. Biol. Med.* **89**, 362–364.
15. Stoker, M. & Macpherson, I. (1964) *Nature* **203**, 1355–1357.
16. Strohl, W. A., Rouse, H., Teets, K. & Schlesinger, R. W. (1970) *Arch. Gesamte Virusforsch.* **31**, 93–112.
17. zur Hausen, H. (1973) *Prog. Exp. Tumor Res.* **18**, 240–259.
18. Eagle, H. (1959) *Science* **130**, 432–437.
19. Bablanian, R., Eggers, H. J. & Tamm, I. (1965) *Virology* **26**, 100–113.
20. Rosenwirth, B., Tjia, S., Westphal, M. & Doerfler, W. (1974) *Virology* **60**, 431–437.
21. Doerfler, W., Lundholm, U. & Hirsch-Kauffmann, M. (1972) *J. Virol.* **9**, 297–308.
22. Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182.
23. Wyatt, G. R. & Cohen, S. S. (1953) *Biochem. J.* **55**, 774–782.
24. Randerath, K. & Struck, H. (1961) *J. Chromatogr.* **6**, 365–367.
25. Randerath, K. (1965) *Nature* **205**, 908.
26. Scheit, K. H. (1966) *Chem. Ber.* **99**, 3884–3891.
27. Broom, A. D., Townsend, L. B., Jones, J. W. & Robins, R. K. (1964) *Biochemistry* **3**, 494–500.
28. Eidinoff, M. L., Reilly, H. C., Knoll, J. E. & Marrian, D. H. (1952) *J. Biol. Chem.* **199**, 511–516.
29. Fink, R. M. & Fink, K. (1962) *J. Biol. Chem.* **237**, 2889–2891.
30. Rubery, E. D. & Newton, A. A. (1973) *Biochim. Biophys. Acta* **324**, 24–36.
31. Ortin, J., Scheidtmann, K. H., Greenberg, R., Westphal, M. & Doerfler, W. (1976) *J. Virol.* **20**, in press, November.
32. Nass, M. M. K. (1973) *J. Mol. Biol.* **80**, 155–175.
33. Kaye, A. M. & Winocour, E. (1967) *J. Mol. Biol.* **24**, 475–478.
34. Eskin, B., Lautenberger, J. A. & Linn, S. (1973) *J. Virol.* **11**, 1020–1023.
35. Herrlich, P., Rahmsdorf, H. J. & Schweiger, M. (1973) *Adv. Biosci.* **12**, 523–537.
36. Pfennig-Yeh, M., Altendorf, K. H., Ponta, H., Hirsch-Kauffmann, M., Herrlich, P. & Schweiger, M. (1976) *Z. Physiol. Chem.* **357**, 328.