The Influence of Arginine Starvation on the Synthesis of Virus High Molecular Weight DNA in HeLa Cells Productively Infected by Adenovirus Type 5

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SUMMARY

In culture cells productively infected by adenovirus a high mol. wt. form of DNA is synthesized which is known to represent, at least in part, virus DNA integrated into cellular DNA. We found that the synthesis of this high mol. wt. DNA and the other DNA size classes can strongly and differentially be influenced by altering the metabolic state of the cells. The effects of different rates of cell growth were tested in this respect as well as arginine deprivation as opposed to application of complete growth medium. Synthesis of virus high mol. wt. DNA and unit genome length DNA is enhanced in actively growing as compared to resting Ad5-infected HeLa cells. Under arginine deficiency, in resting Ad5-infected HeLa cells, integration of virus DNA sequences into cellular DNA is almost totally suppressed whereas virus unit genome length DNA is still synthesized. This differential effect is interpreted by the hypothesis that the formation of virus high mol. wt. DNA is a synthetic process that is independent of the unit size virus DNA replication, but coupled to the synthesis of a special form of cellular DNA that is less effectively shut off by the infection than cellular DNA in general.

INTRODUCTION

The analysis of DNA from KB cells productively infected with adenovirus type 2 (Ad2) by sedimentation into alkaline sucrose gradients revealed the existence of high mol. wt. DNA, containing or consisting of virus DNA integrated into cellular DNA (Burlingham & Doerfler, 1971; Doerfler et al. 1971; Burger & Doerfler, 1974). This form of virus DNA has been elaborately characterized (Burger & Doerfler, 1974; Doerfler et al. 1974; Schick et al. 1976; Fanning & Doerfler, 1977; Baczkó et al. 1978; Fanning et al. 1978; Tyndall et al. 1978). This integrated virus DNA form is still demonstrable if the synthesis of virus unit genome length DNA is suppressed by infection with u.v.-irradiated virus (Fanning et al. 1978) or with DNA- mutants (Tyndall et al. 1978). The formation of the integrated form of the adenovirus DNA is particularly dependent on cellular DNA synthesis; conversely, the synthesis of the virus high mol. wt. DNA is dependent on the m.o.i., whereas synthesis of the virus unit genome length DNA is independent (Fanning & Doerfler, 1977; Fanning et al. 1978) above a m.o.i. of 5. This study describes the differential dependence of the synthesis of the DNA size classes in HeLa cells infected with adenovirus type 5 (Ad5) on the presence of arginine in the culture medium. The existence of a DNA class, containing virus DNA sequences covalently linked to cellular DNA, was confirmed for this virus–cell system.

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Arginine deficiency exerts a differential restraint on the synthesis of virus high mol. wt. DNA as compared to the replication of the virus unit size DNA and this effect is modulated by the state of growth of the monolayer cells. This diverse influence by the same condition corroborates the model that the event leading to the formation of covalent bonds between virus and cellular DNA sequences is independent and different in nature from the process of replication of virus itself though concomitant with cellular DNA synthesis. Pieces of parental virus DNA (Fanning et al. 1978) rather than low mol. wt. forms of newly synthesized adenovirus DNA (Fanning & Doerfler, 1977) are, via a recombination event, integrated into a newly synthesized form of cellular DNA that sediments in the 40 to 90S range and is not subject to the shut-off of host DNA synthesis.

METHODS

Cells. The HeLa strain used in all the experiments was obtained from Flow Laboratories (Irvine, Scotland). Some experiments have been repeated with HeLa, strain Saarbruecken and with KB cells (CCL17), originally from the American Type Culture Collection. The cells were grown in Eagle's minimal essential medium (MEM) supplemented with non-essential amino acids and 2 to 5 % calf serum. In MEM(-arg), arginine was omitted from the MEM. For suspension cultures, the spinner modification of MEM was used.

Virus. Human adenovirus type 5 (strain Ad75) was used throughout. Virus was purified by a newly developed technique, using a potassium bromide gradient with a glycerol layer (Lentfer & Conde, 1978). 14C-thymidine-labelled virus was prepared by the method of Doerfler (1970). The unit of virus concentration used is TCID50 as determined by endpoint titration (Wigand & Kümel, 1977).

Solutions. PBS is phosphate-buffered saline (Dulbecco & Vogt, 1954), PBSd is PBS without Mg2+ and Ca2+. Balanced salt solution (BSS) contains the inorganic salts plus glucose in the same concentrations as in MEM.

DNA analysis by zonal sedimentation. DNA analysis by zonal sedimentation in alkaline sucrose density gradients was performed according to McGrath & Williams (1966) and modified by Doerfler (1969) with minor variations. The cells to be analysed were washed three times with PBS and twice with PBSd and suspended in PBSd at a concentration of 10⁶ cells/ml. Then, 30000 cells were layered on top of a 7 to 18 % sucrose density gradient in 0.3 M-NaOH, 0.7 M-NaCl, 0.005 M-EDTA (p = 1.8) and contained a top layer of 0.33 ml of 0.5 M-NaOH, 0.01 M-EDTA. The cells lysed in the top layer at 4 °C for 18 h. Ultra-centrifugation was for 3 h at 35000 rev/min and 4 °C. The gradients were fractionated from the bottom and a portion of each fraction was dried on to a paper filter, washed with TCA and counted in standard scintillation fluid.

Buoyant density analysis of DNA in pre-formed alkaline CsCl gradients. The fractions of an alkaline sucrose gradient comprising a particular DNA size class were pooled and the buoyant density analysed on pre-formed alkaline CsCl gradients. In a nitrocelulose tube of the Beckman SW40 rotor, a 1 ml cushion of alkaline CsCl (ρ = 1.8) in 0.7 M-NaCl, 0.3 M-NaOH, 0.01 M-EDTA was overlaid with 6 ml of a CsCl gradient (ρ = 1.67 to 1.74) in 0.7 M-NaCl, 0.3 M-NaOH, 0.01 M-EDTA. Samples of the pooled fractions of the alkaline sucrose gradients containing equal amounts of radioactivity were loaded on to this gradient and the tube filled with paraffin. Centrifugation was for 48 h at 20000 rev/min at 10 °C.

DNA–DNA hybridization. The method of Denhardt (1966) was used. The fractions indicated by the bars in Fig. 3 and 4 were pooled, neutralized with citric acid, diluted with an equal volume of water to reach the ionic strength of 3 × SSC, sonicated, held in a boiling water bath for 10 min and incubated in the Denhardt (1966) mixture for hybridization. Aliquots of the samples were acid-precipitated to determine the input radioactivity. A 10 μg amount of denatured Ad5 DNA was immobilized on the filters.
RESULTS

Influence of arginine starvation on the kinetics of the DNA synthesis in uninfected HeLa cells

In order to rule out an unspecific influence of arginine deficiency on DNA synthesis in HeLa cells in general, we studied the fate of newly synthesized, pulse-labelled DNA molecules in uninfected HeLa cells during various chase periods. Non-confluent HeLa cells were pulsed for 15 min with 50 μCi/ml methyl-3H-thymidine, washed thoroughly with BSS and exposed to MEM or MEM(−arg) for the times indicated in Fig. 1. Cell lysis and analysis of the DNA by zonal sedimentation in alkaline sucrose gradients were performed as described in Methods.

As can be concluded from the absorbance graph, the main part of the cellular DNA sedimented rapidly onto the CsCl cushion at the bottom of the tube (referred to as ‘> 100S DNA’ in this study). A second optically discernible part was found around fractions 9 to 10 of the gradients (‘90S DNA’). Newly synthesized DNA appeared as 3H-thymidine incorporated into alkali-stable molecules within the pulse time of 15 min (Fig. 1a). In HeLa cells held under complete MEM, little or no material was labelled within the pulse time that would sediment onto the CsCl cushion. After a chase of 1 h (Fig. 1c), short DNA pieces sedimenting at lower S values than Ad5 unit length marker DNA (34S) were less conspicuous than in cells without chase (Fig. 1a) or after a short chase (Fig. 1b). A broad heterogeneous peak was seen in the approx. range of 40 to 90S and a considerable part of the label incorporated into alkali-stable molecules during the pulse was converted to 90S DNA and > 90S DNA. This sedimentation pattern did not change if longer chase periods were used.

In HeLa cells held under MEM(−arg) (Fig. 1) a similar mode of conversion of slowly sedimenting DNA pieces to material of higher mol. wt. could be traced only with considerable delay. A chase of 1 h was needed to reach a similar sedimentation pattern already found in unstarved cells after a chase period of 15 min. As far as can be deduced from pulse-chase experiments, in uninfected cells DNA replication followed the same pattern under arginine starvation as under complete MEM.

Influence of arginine deficiency on the onset of virus DNA synthesis in Ad5-infected HeLa cells

The delay in the conversion of newly synthesized low mol. wt. DNA into molecules of higher sedimentation value effected by the arginine starvation in uninfected cells might, in infected cells, lead to an asynchrony of the period of virus DNA synthesis in the virus replication cycle. It was therefore necessary to establish the kinetics of the DNA synthesis in arginine-starved and non-starved Ad5-infected cells (Fig. 2). Obviously there is no significant delay in the onset of virus DNA replication, and the time of maximal synthesis of DNA seems to be the same for both media.

DNA synthesis under conditions of arginine deprivation in HeLa cell monolayers infected with Ad5

Resting HeLa cells (confluent monolayers)

HeLa cells grown on a glass surface to confluency (5 × 10⁵ cells/cm²) were washed with BSS thoroughly and the medium changed to MEM or MEM(−arg), both without calf serum. Two hours after the medium change the cultures were washed with the appropriate medium and inoculated with Ad5 at a m.o.i. of 100 TCID₅₀/cell, or else mock-infected. The cells were pulsed 15 to 17 h p.i. with 50 μCi/ml methyl-3H-thymidine, washed twice
Fig. 1. Influence of arginine starvation on the conversion of pulse-labelled DNA into faster sedimenting molecules during a chase period. Uninfected, actively growing HeLa monolayer cells were brought to fresh MEM (---) or MEM(-arg) (■■■■) subjected to a 15 min pulse with 3H-thymidine, quickly washed with the respective medium, lysed and analysed in an alkaline sucrose gradient as described in Methods either (a) directly or after a chase of (b) 15 min, (c) 1 h, (d) 4 h or (e) 16 h. Sedimentation is from right to left. The $A_{254}$ (----) was very similar for all gradients and has therefore been included in (a) only. † Position of Ad5 marker DNA (34S); • CsCl cushion.
DNA size classes in adenovirus-infected cells

Fig. 2. Influence of arginine deficiency on the rate of DNA synthesis in the virus replication cycle. Non-confluent monolayers of HeLa cells were subjected to MEM (●—●) or MEM(-arg) (△—△) both without calf serum and infected 8 h later with Ad5 at a m.o.i. of 200. For each indicated time p.i., three Petri dishes were pulsed for 30 min with 50 μCi 3H-thymidine per ml of the respective medium. The cells were washed, collected and fragmented by freezing and thawing. In an aliquot the protein concentration was determined by the biuret method after removal of debris by centrifugation (10 min, 200 g). The radioactivity of the TCA-precipitable material is given as specific radioactivity (3H ct/min/μg protein) to account for differences in the number of cells harvested from the Petri dishes.

with PBS and twice with PBSd, lysed and the DNA analysed by zonal sedimentation in an alkaline sucrose gradient as described in Methods (see Fig. 3). The DNA sedimenting at lower S values than the virus marker DNA from purified 14C-labelled virions (34S) has not been dealt with in this study. The main part of the DNA newly synthesized during the 3H-thymidine pulse 15 to 17 h p.i. in Ad5-infected cells, sediments in the three size classes 34S, 90S and the high mol. wt. virus DNA (40 to 90S). This size class has been shown to contain virus DNA integrated by covalent linkage into cellular DNA in Ad2-infected KB cells (Burger & Doerfler, 1974) and, by some criteria, in Ad2-infected HeLa cells (Baczko et al. 1978). We have extended this study and confirmed that the virus high mol. wt. DNA in Ad5-infected HeLa cells indeed contains virus DNA sequences joined to cellular sequences by covalent bonds (see later). Some insight into the conditions of the synthesis of this DNA form may be gained from the observation shown in Fig. 3d that the synthesis of the various virus DNA size classes is influenced very differently by one and the same metabolic condition. In confluent HeLa cells that had been infected with Ad5 under arginine starvation, virus unit size DNA is synthesized but the high mol. wt. form of virus DNA can no longer be demonstrated. DNA that is newly synthesized in these cells 15 to 17 h p.i. and sediments at 40 to 90S is reduced, but still prominent (Fig. 3d), when compared to the 40 to 90S stratum of the controls (Fig. 3b and c). If however, the fractions covering the 40 to 90S range were pooled and hybridized to virus DNA immobilized on nitrocellulose filters, no significant amount of label bound to the filter (Table 1). The total amount of DNA synthesized in arginine-starved cells within the pulse time is diminished to approx. 40% of the amount in unstarved infected cells (mean of four experiments). Considering the delay in the processing of newly synthesized DNA under arginine deficiency, we also used later pulse times (17 to 21 h p.i.) Two different HeLa cell lines and KB cells were tested. All these variations led to the same result (data not shown). In uninfected cells (Fig. 3a, b) a specific reduction of the synthesis of cellular 40 to 90S DNA was not observed; arginine starvation reduced the 90S class and the 40 to 90S DNA by about the same ratio. Instead of using media without serum, in a
Fig. 3. Effect of arginine deficiency on the synthesis of the DNA size classes in confluent HeLa cells. HeLa cell monolayers of high cell density (5 × 10⁶/cm²) were subjected to a medium change to MEM (a, c) or MEM(-arg) (b, d). 8 h later the cells were mock infected (a, b) or infected (c, d) with Ad5 at a m.o.i. of 100 TCID₅₀/cell, pulsed 14 to 17 h p.i. (or post mock infection) with 30 µCi/ml medium of ³H-thymidine, washed, lysed and analysed as described in Methods. The fractions of the gradients covered by the bars were pooled and hybridized to membrane filters to which 10 µg Ad5 DNA per filter had been immobilized. Sedimentation is from right to left. ‡, Position of Ad5 marker DNA.

Table 1. Amount of virus DNA sequences in the 40 to 90S size class isolated from growing HeLa cells infected with Ad5 under MEM or MEM(-arg)*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Radioactivity of the pool</th>
<th>Hybridization to Ad2 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ct/min</td>
<td>% Total ct/min on the gradient</td>
</tr>
<tr>
<td>MEM</td>
<td>11755</td>
<td>18.1</td>
</tr>
<tr>
<td>MEM(-arg)</td>
<td>4652</td>
<td>8.2</td>
</tr>
</tbody>
</table>

* HeLa cells were treated in the same way as described in Fig. 3. The fractions comprising the 40 to 90S range were pooled and hybridized (see Methods). Empty filter binding values (14 %) have been subtracted.

parallel experiment we used MEM and MEM(-arg) containing 2 % calf serum that had been extensively dialysed against BSS; this variation also gave similar results.

Depending on the m.o.i. used, only small amounts of cellular DNA (90S DNA and DNA sedimenting on to the CsCl cushion) were synthesized in HeLa cells 14 to 17 h p.i. and consistently the 90S peak is just apparent in Fig. 3(c). It is remarkable that Fig. 3(d) shows a more prominent 90S peak. Obviously there is more cellular DNA synthesis in arginine-
Fig. 4. Effect of arginine deficiency on the synthesis of the DNA size classes in non-confluent HeLa cell monolayers. Actively growing HeLa monolayer cells (cell density $2 \times 10^5$ cm$^{-2}$) were treated as described in the legend to Fig. 3.

starved cells. Several explanations for this phenomenon seem possible. The possibility that it is merely simulated by a delay in the shut-off of the cellular DNA synthesis was rejected (data not shown). It is possible that the cell responds to the chromosome breaks imposed on it by the arginine deficiency (Freed & Schatz, 1969) by an increased repair synthesis of cellular DNA. Chromosomal DNA synthesis of the cell, cellular repair synthesis and the synthesis of virus DNA in adenovirus-infected cells is effected by different enzyme systems (Bolden et al. 1975; Ito et al. 1975, 1976; Brison et al. 1977). Thus it seems conceivable that arginine deficiency as an inhibiting condition influences the respective synthetic processes differently.

Actively growing HeLa cells (non-confluent monolayers)

If the same experiments were carried out with HeLa cells growing on a glass surface in the state of rapid cell division (cell density $1.5 \times 10^5$ cm$^{-2}$), arginine deficiency could be
Fig. 5. Buoyant density analysis of the virus high mol. wt. DNA. The fractions of the alkaline sucrose gradients covering the 40 to 90S virus high mol. wt. DNA were carefully pooled; samples of these pools containing equal amounts of radioactivity were layered on to a pre-formed shallow alkaline CsCl gradient and sedimented to the buoyant density positions of the DNA components (b, c, d; for experimental conditions see Methods). In a second run, aliquots of the same DNA samples were analysed in the same way after ultrasonic fragmentation (f, g, h). In each run, a mixture of 14C-labelled Ad5 virion DNA and 3H-labelled HeLa cell DNA served as control either without (a) or after ultrasonic treatment (e). In each gradient the refractive index and the radioactivity of the fractions were determined. As the graph for the refractive index (n_2) indicated, the pre-formed CsCl gradient reproducibly stayed fairly linear during centrifugation. The n_2 values were very similar for all gradients and are therefore drawn only in (a) and (e). (a, e) 14C-labelled Ad5 virion DNA and 3H-labelled HeLa cell DNA: •—•, 14C; ▲—▲, 3H. (b, f) Virus high mol. wt. DNA from non-confluent HeLa cells infected under MEM (note intermediate density position, liberation of DNA in the density position of virus DNA upon fragmentation (f)). (c, g) Virus high mol. wt. DNA from non-confluent HeLa cells infected under MEM(−arg) (same result as b, f). (d, h) 40 to 90S DNA from confluent HeLa cells infected under MEM(−arg) [note no DNA in the density position of virus DNA is liberated upon fragmentation (h)], unfragmented DNA (d) shows the density position of cellular DNA.
shown to have a considerably different influence on the relative amounts of DNA in the various size classes compared to confluent cells. In infected cells (Fig. 4c, d) the 40 to 90S DNA is significantly more conspicuous and arginine starvation does not reduce it to any great extent under these conditions. The relatively high efficiency of hybridization to filter-bound Ad5 DNA shows that it is the virus rather than the cellular moiety of this size class which is observed in increased amounts in rapidly growing cells, whether they be starved of arginine or not. Virus unit length DNA, on the other hand, is much more reduced by the condition of arginine deficiency in rapidly growing than in resting monolayer cells. As in resting cells, the 90S DNA size class is demonstrable in arginine-starved, infected cells, while it is suppressed in infected cells under complete MEM (shut-off of host cell DNA synthesis).

Buoyant density analysis of the high mol. wt. virus DNA

In order to identify the high mol. wt. DNA as an integrated DNA form, we determined the buoyant density of the high mol. wt. DNA from HeLa cells infected with Ad5 in MEM or MEM(-arg). To improve the resolution of this method, we used relatively shallow pre-formed alkaline CsCl gradients. Fig. 5 shows the results of this experiment. The DNA from the 40 to 90S stratum from alkaline sucrose gradients (same experiment as described above) was analysed either directly or after ultrasonic fragmentation. Fig. 5(b and f) show the analysis of the high mol. wt. virus DNA from Ad5-infected non-confluent cells held under complete MEM. The high mol. wt. DNA bands at a density position intermediate to cellular and virus marker DNA (Fig. 5b); after sonification (Fig. 5f) it shifts to the positions of virus marker DNA and cellular DNA. The high mol. wt. DNA from growing, infected HeLa cells held under MEM(-arg) shows a similar density, and the same density shift after fragmentation as the high mol. wt. DNA from growing infected cells held under complete MEM. The 40 to 90S DNA from resting HeLa monolayer cells infected with Ad5 under MEM(-arg), however, shows the buoyant density of cellular DNA and does not shift significantly after fragmentation (Fig. 5d, h). These data confirm the notion that the virus DNA sedimenting at 40 to 90S in Ad5-infected HeLa cells indeed contains virus DNA covalently bound to cellular sequences and the virus high mol. wt. DNA synthesized under arginine deprivation in growing cells is of the same nature. The 40 to 90S DNA, newly synthesized in resting HeLa cells (confluent monolayers; Fig. 3d), infected with Ad5 under arginine starvation does however contain virus DNA in minute amounts, if at all; it shows the buoyant density of cellular DNA (Fig. 5d), it does not release DNA of the density of virus DNA upon ultrasonic fragmentation (Fig. 5h) and does not significantly hybridize to Ad5 DNA (Fig. 3d and Table 1).

DISCUSSION

Probably more information would have been available about virus DNA, if an inhibitor had been found that would selectively inhibit the synthesis of only some of the DNA size classes in the infected cell; no such substances have been detected so far (W. Doerfle, personal communication; G. Kümel & H. J. Hammer, unpublished data). Instead, arginine deficiency, as the inhibiting condition, was studied in this respect. As to toxicity, reversibility and specificity, arginine deficiency can be considered to meet the requirements of an inhibitor as a biochemical tool. When evaluating arginine starvation as a condition inhibiting virus synthesis in a rather controlled way, it is important to remember that the starved cells are still actively synthesizing proteins due to an enhanced protein turnover (Wigand & Kümel, 1978). Many, if not all of the virus proteins are being formed in arginine-starved, infected cells (Everitt et al. 1971; Rouse & Schlesinger, 1972; Prage & Rouse, 1976).
As in Ad2-infected KB cells DNA from Ad5-infected HeLa monolayer cells can be separated into size classes by sedimentation in alkaline sucrose gradients. The main part of the cellular DNA present in Ad5-infected or uninfected HeLa cells appears in two optically discernible sedimentation zones in these gradients (Fig. 1), namely DNA sedimenting on to the CsCl cushion (‘> 100S DNA’), another part sedimenting at a value of approx. 90S. The existence of these two cellular DNA forms and the notion that in uninfected cells newly synthesized DNA can be chased from the 40 to 90S stratum into 90S DNA, but not readily into the > 100S DNA (Fig. 1) is apparently not unique for HeLa cells (Burger & Doerfler, 1974; Schick et al. 1976; Tyndall et al. 1978). Newly synthesized DNA is mainly separated into four size classes in Ad5-infected cells (Figs. 3 and 4): virus unit length genome DNA (34S), DNA sedimenting at the position of the 90S form that is predominantly labelled in a 2 h pulse in uninfected cells, 40 to 90S DNA and short DNA pieces (< 34S DNA). Hybridization to Ad5 DNA shows that 90S DNA contains very little if any virus DNA (15 to 17 h p.i.), whereas the 40 to 90S DNA hybridizes with high efficiency to the adenovirus DNA. The buoyant density analysis (Fig. 5) confirms that the 40 to 90S DNA from Ad5-infected HeLa cells indeed contains virus DNA integrated into cellular DNA. If virus high mol. wt. DNA is sonicated a considerable part of the fragment sediments at the cellular DNA density position (Fig. 5f, g) indicating that synthesis of the cellular 40 to 90S size class is still going on, while the synthesis of the cellular 90S size class is already subject to the shut-off by the adenovirus infection. This is consistent with published work: Tyndall et al. (1978) concluded that incorporation of 3H-thymidine into virus high mol. wt. DNA appears to be mainly due to replicating cellular DNA. Schick et al. (1976) have shown the formation of virus high mol. wt. DNA to occur when synthesis of virus DNA sequences has not yet started. The parental virus DNA was not labelled in these experiments; the incorporation of radio-labelled thymidine into the virus high mol. wt. DNA therefore proves the cellular, not the virus moiety of the virus high mol. wt. DNA to be due to de novo synthesis. The same consideration applies to the experiments with u.v.-irradiated virus (Fanning et al. 1978) and DNA- mutants (Tyndall et al. 1978).

The experiments with starved cells suggest that arginine deficiency exerts a differential effect on the formation of the various DNA size classes in the infected cell. In cells grown to dense monolayer, and therefore being in a steady state of growth with reduced replication of cellular DNA, if infected with Ad5 under arginine deficiency, integration of virus DNA into the 40 to 90S class can be demonstrated to only a limited degree (Fig. 3d and Table 1). These results of the sedimentation and hybridization experiments have been confirmed by the buoyant density analysis in alkaline CsCl gradients (Fig. 5d, h). On the other hand virus unit size DNA is still synthesized in a quantity of about 40% of the amount demonstrable in unstarved, resting cells (Fig. 3c, d).

In non-confluent monolayer cells that are still in the phase of rapid cell division and actively synthesizing cellular DNA the 40 to 90S class is more prominent 15 to 17 h p.i. under complete MEM. Arginine starvation in these cells has a quite different effect (Fig. 4c, d): it does not suppress integration of virus sequences into the 40 to 90S class significantly; the virus DNA sedimenting at 34S, however, is reduced drastically to values below 10% of the amount in unstarved growing cells at the maximum of DNA synthesis 14 to 17 h p.i.

This differential restraint on the synthesis of the virus high mol. wt. DNA, the virus 34S DNA and presumably the cellular 90S DNA in Ad5-infected HeLa cells by the same condition namely arginine deficiency in different states of growth of the cell leads to the following hypothesis: the constitutive event in the formation of integrated virus DNA is independent of and different in nature to the synthesis of virus unit size DNA. It depends on the concurrent synthesis of a 40 to 90S cellular DNA form (the synthesis of which is not effectively blocked by the host cell DNA shut-off) but not on the synthesis of the other forms of cellular
DNA size classes in adenovirus-infected cells

DNA (90S, > 100S). The formation of the virus high mol. wt. DNA might eventually be due to the action of enzymes or regulation processes different from those acting in the synthesis of virus 34S DNA, cellular DNA forms sedimenting at 40 to 90S and cellular 90S DNA. The evidence (Ito et al. 1975, 1976; Brison et al. 1977) that different enzymes appear to be engaged in the synthesis of cellular and adenovirus DNA replication and in DNA repair synthesis corroborates this idea. This hypothesis would be consistent with the postulation (Fanning & Doerfler, 1977), that the high mol. wt. forms of virus DNA arise as a consequence of legitimate or illegitimate recombination and with the observation that some parts of the virus genome are over-represented in the integrated form of the virus DNA. This hypothesis would further solve an apparent contradiction in published work: the production of virus high mol. wt. DNA is particularly dependent on cellular DNA synthesis although enhanced in cells infected with high m.o.i. (Fanning & Doerfler, 1977; Fanning et al. 1978) while the cellular DNA synthesis is known to be most effectively shut off in cells infected at high m.o.i.

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