In Vitro Growth Control Phenotypes of Transformed Rodent Cells prior to and following Tumorigenesis

Frank J. O’Neill and Laurence Renzetti
Department of Cellular, Viral, and Molecular Biology, University of Utah and Research Service, VA Medical Center, Salt Lake City, Utah 84148

ABSTRACT

A number of virus and chemical carcinogen-transformed cell lines were generated in tissue culture and analyzed for growth control phenotypes prior to and following tumorigenesis in appropriate hosts. The cell lines include those of mouse, rat, human, and Syrian hamster, transformed by papovaviruses and adenoviruses (DNA) or murine (RNA) tumor viruses. Cell lines were assayed for: (a) multinucleation or uncontrolled nuclear division (UND+) and uncontrolled DNA synthesis in cytochalasin B (CB) medium; and (b) the continuation of DNA synthesis in media containing reduced (0.5%) amounts of serum. All or nearly all lines of DNA virus transformants exhibited UND+ and high frequencies of DNA-synthetic cells in CB medium. Two lines of SV40-transformed hamster cells also showed UND+ following tumorigenesis in weanling hamsters. In addition, DNA virus transformants showed the ability to continue DNA synthesis unabated in low-serum medium. In contrast, the mouse sarcoma virus (MSV)-transformed lines exhibited varying degrees of controlled nuclear division and reduced DNA synthesis in CB medium, both prior to and following tumorigenesis. However, the reduction in DNA-synthetic cells was not as great as that found in untransformed cells. Results similar to the RNA virus transformants were observed with hamster cells transformed by chemical carcinogens. Nearly all of the MSV-transformed lines showed significantly reduced levels of DNA synthesis in low-serum medium as was found in untransformed cells. One cell line, KA31, was followed through three consecutive in vivo tumorigenic passages, but these cells did not acquire UND+. In addition, the glioblastoma cell lines not show UND+. In addition, the glioblastoma cell lines and adenoviruses (DNA) or murine (RNA) tumor viruses. Cell lines were also used. These include BALB/c 3T3 cells, HaEF, MEF, REF, and human fetal lung fibroblasts. Most cell lines were cultured in Eagle’s minimum essential medium and maintained in Eagle’s minimum essential medium with 5% bovine serum. In the CB and high-serum experiments, fetal bovine serum was used at 10%; for cells treated with low serum, 0.5% fetal bovine serum was used.

INTRODUCTION

Virus and chemical carcinogen-transformed cells and cultures of tumor cells have been shown to exhibit uncontrolled growth by a variety of criteria or phenotypes (1–27). It has been shown that many lines of transformed cells become highly multinucleated in the presence of low levels of CB2 which prevents cytoplasmic cleavage (2, 7, 8, 15, 19, 28). The multinucleated phenotype has been termed UND+, although the effect of CB on DNA synthesis has not always been analyzed (15, 17, 28, 29). It has been shown however, that some human glioblastoma cell lines (15, 16) and some RNA tumor virus transformants, in contrast to DNA virus transformants, do not show UND+ (16, 19). In addition, the glioblastoma cell lines and RNA virus transformants, unlike DNA virus transformants, are growth arrested by caffeine or theophylline treatment (16) or by incubation in low-serum-containing medium (6).

It has been suggested that UND+ might represent a characteristic feature of oncogenic transformation (15, 28). This suggestion is significant since there is the observation that UND+ is a reflection of uncontrolled DNA synthesis (15), which could explain the uncontrolled growth of tumor cells in vivo. Some reports, however, have not shown an invariable association between transformation and CB-induced multinucleation (14–16, 18, 19, 25, 30). The present report has analyzed 2 of the above growth properties, prior to and following in vivo passage, of a variety of transformed rodent cell lines. Many of the RNA virus-transformed lines which fail to show UND+ showed reduced DNA synthesis in CB medium and are G1-arrested in low serum. These cell lines, nevertheless, are tumorigenic. The cultured tumors often do not exhibit UND+, and they continue to be G1 arrested in low-serum medium.

MATERIALS AND METHODS

Cell Lines. The DNA and RNA virus-transformed cell lines are shown in Table 1 along with the species of origin and their responses to CB. In the case of RNA virus transformants, Table 1 shows whether they produce transforming viruses. A number of untransformed cell lines were also used. These include BALB/c 3T3 cells, HaEF, MEF, REF, and human fetal lung fibroblasts. Most cell lines were cultured in Eagle’s minimum essential medium and maintained in Eagle’s minimum essential medium with 5% bovine serum. In the CB and high-serum experiments, fetal bovine serum was used at 10%; for cells treated with low serum, 0.5% fetal bovine serum was used.

CB Assay. Cells were seeded into 25-sq-cm Petri dishes containing 22-sq-mm coverslips with normal medium (10% serum) as described (14, 16). On the following day, the cultures were incubated in medium containing CB (1.5 or 2.0 µg/ml; hamster cells often required 3.0 µg/ml), harvested, and analyzed for multinucleation as described (16). Cultures were refed every second or third day.

Cellular DNA synthesis was monitored in CB-treated and untreated cultures until Day 7 by incubating replicate cultures with [3H]thymidine (1 µCi/ml) for 1 hr prior to harvesting. Cultures were always refed within 24 hr prior to incubation in [3H]thymidine. The cultures were then fixed and processed for autoradiography as described (14). The densities of untreated 7-day cultures were 5– to 10-fold greater than those of CB-treated cultures (data not shown). In some experiments, DNA synthesis was also monitored following a 7-day CB treatment schedule when the CB was washed out and the cultures were incubated in normal medium. Replicate cultures were then periodically incubated in [3H]thymidine medium, and the possible reappearance of DNA-synthetic cells was followed as a function of time after reversal by auto-

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radiography. All lines showing uncontrolled nuclear division were referred to as UND*, and those showing controlled nuclear division were referred to as UND-. Cells showing UND- contained at least 50% binucleated and trinucleated cells and not more than 10% with 4 or more nuclei. Cell lines were classified as UND* when more than 35% of the cells showed 4 or more nuclei.

**Growth in Low-Serum Medium.** Replicate cultures were incubated in 10 or 0.5% fetal bovine serum for 7 days during which they were refed every 2 or 3 days. Periodically, DNA synthesis was measured autoradiographically, as described above. At Day 7, the densities attained by low-serum-treated cultures were 5- to 10-fold less than those of the high-serum-treated cultures (data not shown). Cultures to be reversed from the low-serum treatment were incubated in 10% serum, and DNA synthesis was monitored, again by autoradiography. These experiments could not be performed in some cell lines, e.g., KA31, 3T3, and M-NRK, because the monolayers detached either wholly or partially from their substrates after a few days of incubation in 10 or 0.5% serum.

**Tumorigenesis.** Weanling BALB/c mice or weanling outbred Syrian hamsters were given s.c. inoculations of dilutions of tissue culture cells, as described (13). Tumors were identified either visually or by palpation, twice weekly. Inoculated animals were followed for the development of tumors for 4 months.

**Culture of Tumor Cells.** Selected tumors were aseptically excised, minced with sterile scissors, and trypsinized. The freed tumor cells were then incubated in culture medium as described (13). Many cultured tumors, except for MSV-85 and SV40 transformants, were shown to be tumorigenic in appropriate hosts upon a second cycle of inoculation. MSV-85 cells and SV40 transformants were not subjected to this second cycle of tumorigenesis. Tumor cell lines were given the designation IV (for in vivo) following the designation of the cell line. The number following IV indicated the log of the number of cells inoculated, i.e., HaEF/BP clone 6 IV~ is clone 6 of benzopyrene-transformed hamster cells following a single in vivo passage with 105 cells. KA31 was given the designations, KA31 IV* (2x) and KA31 IV* (3x), following the second (2x) and third (3x) serial in vivo passages.

**Rescue of Sarcoma Virus from RNA Virus Transformants.** KA31 and MSV-85 were infected with either Kirsten or Rauscher mouse leukemia virus and incubated for 7 to 10 days. Cell-free filtrates from either Kirsten or Rauscher mouse leukemia virus-infected cells were then allowed to infect BALB/c 3T3 cells or MEF, and transformed foci were usually scored 7 to 10 days later. Cultures were monitored for 3 additional weeks when foci were not apparent within 7 to 10 days.

### RESULTS

- **UND*. The great majority of DNA virus-transformed rodent and human cell lines showed high degrees of UND*. This included SV40, polyoma, and adenovirus type 7 and 12 transformants (Chart 1). Those lines contained at least 50% of cells with 4 or more nuclei following 7 days of CB treatment. CB-treated normal cells usually revealed <5% with 4 or more nuclei; 3T3 was slightly above 5%.

In contrast to the DNA virus transformants, RNA virus transformants showed inhibition of multinucleation in CB medium (UND). Many of the lines contained <10% cells with 4 or more nuclei. KA31, MSV-85, K-NRK, M-NRK, NRK/WSV, and others exhibited low levels of multinucleation, barely above or similar to those of their “normal” parental cell lines (Chart 2). Some lines (MSV-85 and some of the MEF/MSV-Ki transformants) exhibited more multinucleation, somewhat above untrans-
formed cells. Clone 4 of MEF/MSV-Ki was significantly multinucleated as has been reported previously (19). However, these levels of multinucleation were markedly below those found in DNA virus transformants. Chemical carcinogen-transformed cell lines also contained low levels of CB-induced multinucleation or were frankly UND⁺ (Chart 2).

**DNA Synthesis in CB-treated Cells.** Since cell lines with low levels of CB-induced multinucleation are sometimes considered to be uncontrolled (28, 29), we performed simultaneous analyses of cellular DNA synthesis in CB-treated DNA and RNA virus-transformed cells. Since DNA synthesis precedes and is generally required for nuclear division, this allowed for a more fundamental comparison among the different types of transformants. As shown in Chart 3, concentrations of CB (2 to 3 μg/ml) capable of arresting cytoplasmic division and resulting in high degrees of UND⁺ did not affect DNA synthesis in DNA virus transformants, i.e., DNA synthesis occurred in a high percentage of cells throughout the course of CB treatment.

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**Chart 1.** CB-induced multinucleation in untransformed and DNA tumor virus-transformed cells. Cell lines were treated with CB (1.5 to 2.0 μg/ml) for 7 days except for HaEF and its transformants which received CB (3.0 μg/ml). Final 2 graphs, analysis of transformants following tumorigenesis in weanling hamsters. cl, clone; HFL, human fetal lung fibroblasts; NRK, normal rat kidney; Ad7, adenovirus type 7; Ad12, adenovirus type 12.

**Chart 2.** CB-induced multinucleation in RNA tumor virus and chemical carcinogen-induced transformants. Procedures are as in Chart 1. cl, clone.

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**Growth Control Phenotypes and Virus Transformation**
Again, in contrast to the DNA virus transformants, the RNA virus transformants showed greatly reduced frequencies of cell DNA synthesis in CB medium. At 7 days of CB treatment, usually 10% or fewer cells incorporated $[^3H]$thymidine (Chart 4). This occurred even in cell lines which exhibited low levels of UND$^+$ and in cell lines which did not exhibit a marked reduction in DNA-synthetic cells following 7 days in normal medium, e.g., MSV-85 and BALB/MEF/MSV-Ki clone 2 IV$^6$. As shown previously, RNA virus-transformed cells exhibit greatly reduced DNA-synthetic frequencies as they attain saturation density (14, 15). This is again apparent in Chart 4. However, the reduction apparent in the CB treatment curves cannot be attributed to the attainment of saturation density, since CB-treated cells do not divide as long as CB remains in the culture medium. Moreover, we have directly counted CB-treated cells, and their densities at Day 7 are less than or equal to their densities at Day 1. Cells propagated in normal medium for 7 days exhibit densities 5- to 10-fold greater (data not shown).

Some of the CB-treated cell lines appeared to be arrested in early G1, because removal of CB was followed by a burst of DNA-synthetic cells 18 to 24 hr later (Chart 4). Others may have been arrested in G1 or perhaps other cell cycle phases (G2), but removal of CB was not followed by a burst of DNA synthesis during the period studied. These cell lines (clones 2 and 3 of BALB/MEF/MSV-Ki, and to some extent REF/MSV-Ki) may have been irreversibly blocked by CB.

**Effects of Low Serum.** Following incubation in 0.5% serum, all cell lines transformed by DNA viruses continued DNA synthesis in a high percentage of cells (Chart 3). The frequency of DNA-synthetic cells was similar in either 10 or 0.5% fetal serum. An increase in the serum concentration from 0.5 to 10%, following 7 days of incubation, often did not result in a further increase in the frequency of DNA-synthetic cells. In contrast, the great majority of RNA virus transformants exhibited greatly reduced levels of DNA synthesis (Chart 4). Again, these cell lines appeared to be arrested in G1, since incubation in 10% serum was followed by a burst of DNA-synthetic cells. A burst in DNA-synthetic cells also occurred in cells maintained in 10% serum at about 12 to 20 hr following feeding but occurred at reduced magnitudes and not in all cell lines. This burst of DNA-synthetic cells, following serum concentration elevation, occurred in cell lines which did not similarly respond following reversal of CB media to normal media, i.e., MEF/MSV-Ki clones 2 and 3 and REF/MSV-Ki.

**Tumorigenesis.** KA31, MSV-85, HaEF/MSV, and MEF/MSV were RNA virus transformants inoculated s.c. into weanling animals. Two HaEF/SV40 transformants were also inoculated into weanling hamsters, as were 3 lines of carcinogen-transformed hamster cells. As shown in Table 2, the KA31 line and MEF/MSV lines were highly tumorigenic in weanling BALB/c mice. The tumors produced by the MEF/MSV lines (virus producers) were invariably hemorrhagic but were histopathologically classified as sarcomas. The tumors produced by KA31 and HaEF/MSV were solid masses as were those formed by the SV40 transformants. HaEF/MSV and MSV-85 were not highly tumorigenic. HaEF/MSV produced tumors only when 10$^6$ or 10$^7$ cells were inoculated. MSV-85 produced tumors with as few as 10$^3$ cells, but these masses always disappeared within 10 days after their formation. Tumors from SV40 transformants, the carcinogen transformants, KA31, HaEF/MSV, MEF/MSV, and MSV-85 (prior to
disappearance of tumor mass) were surgically excised and propagated in tissue culture. KA31 was also returned to tissue culture following the second and third successive in vivo passages. All tumors could be established in tissue culture readily and propagated continuously.

**In Vitro Phenotypes of Tumor Cells.** In most cases, KA31, HaEF/MSV, MSV-85, and MEF/MSV tumor-derived cell lines were similar to parental cells prior to tumorigenesis in that they exhibited either UND or low levels of UND (Chart 5). Exceptions were MEF/MSV-Ki clone 2 IV7 and MEF/MSV-Ki clone 1 IV7. These lines exhibited moderate levels of UND, although CB continued to significantly inhibit DNA synthesis (Chart 4). Both of these lines propagated to significantly higher densities in normal medium than parental clone 1 and clone 2 cells (data not shown). MEF/MSV-Ki clone 2 IV8, when propagated in normal medium, failed to exhibit a reduction of DNA synthesis at confluency (Chart 4), but CB treatment markedly inhibited DNA synthesis.

The HaEF/MSV-Ki and KA31 cell lines were analyzed following the second and third (KA31 only) in vivo passages (Charts 4 and 5). Except perhaps for KA31 IV (2x), they maintained controlled nuclear division in CB medium and also showed G1 arrest in low-serum medium. In these assays, these cells appeared not unlike untransformed cells. They were arrested in G1 by CB or low-serum medium. KA31 IV6 (2x) showed a significant number of cells with 4 or 5 nuclei and no cells with more than 5 nuclei. This moderate- or low-level UND phenotype was absent in KA31 IV7 (3x) cells.

The kinetics of DNA synthesis following CB withdrawal from these tumorigenic UND derivatives again suggested CB-induced G1 arrest. However, in MEF/MSV-Ki clone 2 IV8, removal of CB was not followed by a burst of DNA-synthetic cells as was also observed in parental clone 2 and clone 3 cells.

Similar results were observed with low-serum treatment. Cultured tumor cells derived from RNA virus transformants exhibited markedly reduced frequencies of cellular DNA synthesis, and raising the serum concentration to 10% was often followed by a burst of DNA-synthetic cells (Chart 4).

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**Table 2**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of cells inoculated</th>
<th>No. of animals with tumors/no. of animals inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSV-85</td>
<td>$10^8$</td>
<td>0/2$^b$</td>
</tr>
<tr>
<td>KA31</td>
<td>$10^6$</td>
<td>0/4$^{ad}$</td>
</tr>
<tr>
<td>KA31, 2nd passage</td>
<td>$10^6$</td>
<td>0/4$^{ad}$</td>
</tr>
<tr>
<td>KA31, 3rd passage</td>
<td>$10^5$</td>
<td>0/4$^{ad}$</td>
</tr>
<tr>
<td>MEF/MSV-Ki clone 1</td>
<td>$10^6$</td>
<td>0/4$^{ad}$</td>
</tr>
<tr>
<td>MEF/MSV-Ki clone 2</td>
<td>$10^6$</td>
<td>0/4$^{ad}$</td>
</tr>
<tr>
<td>MEF/MSV-Ki clone 3</td>
<td>$10^5$</td>
<td>0/4</td>
</tr>
<tr>
<td>MEF/MSV-Ki clone 4</td>
<td>$10^5$</td>
<td>0/4</td>
</tr>
<tr>
<td>HaEF/MSV-Ki</td>
<td>$10^6$</td>
<td>0/2</td>
</tr>
<tr>
<td>HaEF/MSV-Ki</td>
<td>$10^6$</td>
<td>0/4$^{c}$</td>
</tr>
<tr>
<td>HaEF/MSV-Ki</td>
<td>$10^6$</td>
<td>0/4$^{c}$</td>
</tr>
<tr>
<td>HaEF/MSV-Ki</td>
<td>$10^6$</td>
<td>0/4$^{c}$</td>
</tr>
</tbody>
</table>

$^a$ Although up to $10^7$ cells were often inoculated to test for tumorigenicity, this figure represents the lowest number of cells inoculated which produced tumors in at least 50% of animals.

$^b$ Palpable masses were produced at the site of inoculation within 2 to 3 weeks after inoculation, but the masses always disappeared within 7 to 10 days hence. One such tumor mass was excised and established in tissue culture.

$^c$ Cells from this tumor were minced and then inoculated into 2 weanling hamsters. Both inoculations resulted in tumors, but the number of cells inoculated was not determined.

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**Chart 4.** DNA synthesis in CB-treated and low-serum-treated RNA tumor virus-transformed cells prior to and following tumorigenesis. Procedures are as in Chart 3. cl, clone; C, cells in normal medium.
The tumor cell lines derived from carcinogen-induced transformants responded in a manner similar to that of RNA virus transformants. These cell lines exhibited UND− or low levels of UND+ before and after tumorigenesis (Table 1; Charts 2 and 5). DNA synthesis was not analyzed in either CB or low-serum media.

In contrast, the 2 SV40 transformants analyzed maintained high levels of UND+ following tumorigenesis. They were similar to parental cells analyzed prior to tumorigenesis and to other DNA virus transformants (Chart 1).

DISCUSSION

CB-induced multinucleation or UND+ has been reported to be a characteristic feature of DNA virus-transformed cells (7, 8, 16, 18, 19). In contrast, cells transformed by RNA tumor viruses have been reported to exhibit a predominantly binucleated CB response, although there are occasional exceptions (19). Some controversy has appeared as to whether RNA virus-transformed cells are indeed controlled. Some investigators have argued that multinucleation does exist in CB-treated RNA virus transformants (1, 28, 29). Somers and Murphey (28, 29) have reported multinucleation in MSV-transformed rat NRK, mouse 3T3, and human and mink cells following CB treatment. However, the human parental cell line described was tumor derived and already exhibited UND+ (28), and the untransformed mink cell line (the probable substrate for MSV transformation) also exhibits UND+. CB treatment of MSV-transformed NRK and 3T3 cells produced some cells with 3 or 4 nuclei, but there were few cells with 4 or more nuclei (29). MSV-transformed 3T3 cells exhibited levels of CB-induced multinucleation only marginally above those of untransformed 3T3 cells. These studies did not compare these results to data from DNA virus-transformed cells where others have shown large numbers of cells with 6 or more nuclei following CB treatment (7, 8, 18, 19). Even where cells become moderately multinucleated, we argue that nuclear division is limited or controlled. If nuclear division continued in these CB-treated cells, highly multinucleated cells would be formed. Also, no analysis was made of the ability of transformants to continue DNA synthesis in CB medium (29). We believe this type of analysis to be important since it provides a quantitative evaluation of a process upon which the formation of multinucleated cells depends.

In the present report, DNA and RNA tumor virus-transformed cells, along with carcinogen-induced transformants, have been compared further. In addition to analyzing CB-induced multinucleation and CB-induced inhibition of DNA synthesis, we have compared the capacity of both types of transformants and normal cells to continue DNA synthesis in low-serum medium. CB-treated DNA virus (SV40, adenovirus type 7, and adenovirus type 12) transformants continued DNA synthesis in a high percentage of cells, as did untreated cells, and showed high levels of UND+. Incubation in low-serum-containing medium also did not apparently affect DNA synthesis. The results with the MSV transformants were markedly different. Most MSV transformants showed a UND− phenotype or exhibited UND+ at levels barely above those of untransformed cells. Some MSV transformants exhibited CB-induced multinucleation at levels significantly above those found in untransformed parental cells, but those levels were far below what was found in the DNA virus transformants. Most carcinogen-induced transformants also exhibited the UND− phenotype. When DNA

Unpublished observations.
synthesis was analyzed during CB treatment, it was apparent that most of the RNA virus transformants were incapable of continuing DNA synthesis at levels found in untransformed cells incubated at similar densities. In some lines, the cells appeared to be arrested in G₁, since removal of CB was followed by a burst of DNA-synthetic cells. These DNA-synthetic cells occurred following a lag period of 18 to 24 hr. In other transformants, there was a significant inhibition of DNA synthesis by CB, but removal of CB was not followed by a burst of DNA-synthetic cells. In any case, CB markedly reduced the frequency of DNA-synthetic cells, even in lines which exhibited moderate levels of UND+. Since RNA virus-transformed cells usually exhibit only low levels of UND+ or are frankly UND- and show marked inhibition of DNA synthesis, nuclear division and DNA synthesis are limited or controlled. These properties are more apparent when the transformation phenotypes are compared to those of cells transformed by DNA viruses.

It is unlikely that CB reduces DNA synthesis only by some trivial mechanism like inhibition of transport, perhaps [³H]thymidine transport, since: (a) dihydrocytochalasin B, an analogue known not to inhibit transport, yields results very similar to those obtained with CB (data not shown); (b) in CB-treated cultures with UND−, the amount of label in those few cells which are labeled is as great as in cells which are frankly UND-. If transport was significantly involved, many of the cells which are labeled should be labeled with reduced intensity; (c) if CB inhibited [³H]thymidine transport but not DNA synthesis per se, the UND− cell lines would be expected to continue nuclear division in the presence of CB. They of course do not; (d) if CB inhibited transport, removal of CB would be expected to result in an early reappearance of [³H]thymidine incorporation. This was not observed; moreover, some UND− lines could not recover the ability to incorporate the label after CB withdrawal.

What is the relationship of these in vitro parameters of transformation to tumorigenicity? Clearly, cells with UND−, those with low levels of UND+, and those which are growth arrested in low-serum media are nevertheless tumorigenic. The tumorigenicity of these cells does not correlate with the degree of UND+, since cells with low or moderate levels of uncontrolled DNA synthesis are not generally more tumorigenic than are cells with more normal growth control phenotypes. In addition, when tumors are returned to tissue culture, there is no consistent conversion from UND− to UND+ or increase in UND+ from low to high levels. Some clones of MEF/MSV and HaEF/MSV-Ki tumor cells exhibited some elevation in the degree of UND+, but others did not. KA31 cells maintained controlled phenotypes through 3 in vivo passages. This is in contrast to a strain of hamster BHK-21 cells which consistently converts to UND+ following a single in vivo passage (13). The CB and low-serum phenotypes of RNA virus-transformed cells are not completely normal because the frequency of DNA-synthetic cells is somewhat above that of untransformed cells. However, since the difference is often so marginal, when compared to the difference between normal and DNA virus transformants, it is difficult at this time to attach much significance to it. The results of the experiments with 0.5 and 10% serum suggest that the loss of serum dependency is not an essential requirement for tumorigenicity, since a significant number of cultured tumor cells could still be growth-arrested by low serum. These results are apparently in contrast to those of tumor-derived Chinese hamster cells which invariably lose their serum dependency (22). We would argue that in some systems the loss of serum dependence might be required for tumorigenicity while in others it might not. This is analogous to the observation of the in vivo conversion of UND− to UND+ in some strains of BHK-21 cells (13) but not in others or in the systems reported here. These results suggest that, with the current methods of analyses, UND+ and uncontrolled DNA synthesis in the presence of CB or low serum are not reliable in vitro parameters of either neoplastic transformation or tumorigenicity.

These observations provide additional evidence in support of earlier suggestions that, since the transformation phenotypes are different, the mechanisms of transformation used by the 2 groups of viruses are different (14, 16, 18, 19). CB or low-serum treatment of RNA virus transformants abrogates cellular DNA synthesis to various degrees, but similar treatment of DNA virus transformants does not appreciably alter DNA-synthetic levels. It has been argued that the continued presence in transformants of SV40 T-antigen may act to drive cells through the cell cycle, even in CB medium (18). The factors present in RNA virus transformants which maintain transformation without such a driving force behind DNA synthesis remain to be elucidated.

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