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RAPID COMMUNICATIONS

Inducers of DNA Synthesis: Levels Higher in Transformed Cells than in Normal Cells

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ABSTRACT The objective of this study was to determine whether transformed cells have greater DNA synthesis-inducing ability (DSIA) than normal cells when fused with G_1 phase cells. HeLa cells synchronized in G_1 phase, prelabeled with large latex beads, were fused separately with (*a*) quiescent human diploid fibroblasts (HDF), (*b*) HDF partially synchronized in late G_1, and random populations of (*c*) HeLa, (*d*) WI-38, (*e*) SV-40 transformed WI-38, (*f*) CHO, (*g*) chemically transformed mouse cells (AKR-MCA), and (*h*) T98G human glioblastoma cells (all prelabeled with small latex beads) using UV-inactivated Sendai virus. The fusion mixture was incubated with [^{3}H] thymidine, sampled at regular intervals, and processed for radioautography. Among the heterodikaryons, the frequency of those with a labeled and an unlabeled nuclei (L/U) were scored as a function of time after fusion. The faster the induction of DNA synthesis in HeLa G_1, the steeper the drop in the L/U class and hence the higher DSIA in the S phase cells. The DSIA, which is indicative of the intracellular levels of the inducers of DNA synthesis, was the highest in HeLa and virally transformed WI-38 cells and the lowest in normal human diploid fibroblasts (HDF) while those of chemically and spontaneously transformed cells are intermediate between these two extremes. Higher level of DNA synthesis inducers appears to be one of the pleotropic effects of transformation by DNA tumor viruses. These studies also revealed that initiation of DNA synthesis per se is regulated by the presence of inducers and not by inhibitors.

When noncycling senescent human diploid fibroblasts (HDF) are fused with cycling young HDF using UV-inactivated Sendai virus, initiation of DNA synthesis is inhibited in the nuclei of young HDF residing in the heterokaryons (16). If the young HDF nuclei are in S phase at the time of cell fusion, they continue to synthesize DNA in heterokaryons (35). Similar results are obtained when chemically transformed and radiation-transformed human cells and certain other tumor cells are fused with senescent HDF (28, 29). However, when HeLa cells or SV-40-transformed WI-38 (SVWI-38) are fused with senescent HDF, the nuclei of these cells present in the heterokaryons can not only enter S phase but also can induce DNA synthesis in the senescent nuclei (17). Young HDF with a "retarded growth rate" as a result of exposure to sublethal doses of mitomycin C or amino acid analogs (18) and quiescent (G_0) HDF obtained by serum deprivation (21, 30) behave like senescent HDF in inhibiting the initiation of DNA synthesis in young HDF nuclei present in the heterokaryons. These results are explained by assuming the presence of inhibitors of DNA synthesis in noncycling senescent or quiescent cells that are effective on normal HDF and chemically transformed human cells but not on HeLa or SV-40 transformed HDF, which are capable of overriding their inhibitory effects (30). According to this hypothesis, initiation of DNA synthesis is regulated by the presence or absence of an inhibitor.

In contrast, studies from our laboratory indicate that initiation of DNA synthesis in mammalian cells is under positive control, i.e., the presence of inducing factors. The presence of factors that initiate DNA synthesis in S phase cells and their ability to induce this event in G_1 nuclei have been demonstrated by cell fusion (8, 23) and nuclear transplantation (6, 9) studies in various experimental systems. Further studies by Rao et al. (26) indicate that the inducer(s) of DNA synthesis accumulate gradually throughout the G_1 period, reaching a critical concentration at the G_1-S boundary when DNA replication is initiated.

The objective of this study was twofold: (*a*) to determine whether DNA synthesis per se is under positive or negative control or both; (*b*) to provide experimental evidence in support of the hypothesis that malignant (HeLa) and virally transformed (SVWI-38) cells have higher intracellular concentrations of DNA synthesis inducers than normal cells, since malignant cells are able to induce DNA synthesis in noncycling cells after cell fusion. Therefore, we decided to determine the

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relative DNA synthesis-inducing activity (DSIA) in normal, malignant, virally, chemically, and spontaneously transformed cells by studying their kinetics of induction of DNA synthesis in synchronized G_1 phase HeLa cells after cell fusion.

MATERIALS AND METHODS

Cells and Cell Synchrony: In this study, six different cell lines were used. We selected a normal human diploid cell line (WI-38), SV-40-transformed WI-38 (SVWI-38), a human tumor cell line (HeLa), a chemically transformed mouse cell line (AKR-MCA), and a spontaneously transformed Chinese hamster ovary cell line (CHO). We also selected a human glioblastoma cell line (T98G) that behaves like normal HDF with regard to G_1 arrest under stationary phase conditions (27). WI-38 and SVWI-38 cells were obtained from American Type Culture Collection, Rockville, MD. WI-38 cells in the 18th–20th passages were used for these experiments. The 3-methyl-cholanthrene-transformed mouse cell line (AKR-MCA) was a gift from Dr. Harold L. Moses of Mayo Clinic, Rochester, MN. T98G cells were a gift from Dr. Gretchen Stein, University of Colorado, Boulder, CO. HeLa and CHO cells are routinely maintained in our laboratory. All the cell lines except SVWI-38 and T98G were grown in McCoy's 5A medium as monolayer cultures, supplemented with glutamine (1%), antibiotics, and fetal bovine calf serum (10%) at 37°C in a humidified CO_2 incubator. WI-38 cells were grown in the same medium with 20% serum. SVWI-38 and T98G cells were grown in Eagle's minimal essential medium (MEM) with 10% serum and the above-mentioned supplements. HeLa cells were synchronized in mitosis by the N_2O block method (22). Three hours after the reversal of the N_2O block, when >95% of the cells had completed mitosis, these early G_1 cells were used for cell fusion.

Cell Fusion: Random populations of WI-38, SVWI-38, T98G, HeLa, AKR-MCA, and CHO, all labeled with small (0.45-μm diameter) latex beads, were separately fused with synchronized G_1 phase HeLa cells prelabeled with large (0.9-μm diameter) latex spheres using UV-inactivated Sendai virus. The procedures for Sendai virus-induced cell fusion have been described (23). Only the heterodikaryons containing both large and small latex beads were scored for the incorporation of [^{3}H]dThd. As a control, we scored the unfused mononucleate cells of the two parental types for their labeling indices.

RESULTS

Differences in the DNA Synthesis-inducing Activities of Normal and Transformed Cells

After radioautography, the heterodikaryons in the fusion mixtures were classified into three types, i.e., cells with (*a*) both nuclei unlabeled (U/U); (*b*) both nuclei labeled (L/L); and (*c*) one nucleus labeled and the other unlabeled (L/U). The U/U class was derived by the fusion of either G_1 or G_2 phase cells of a random population with HeLa G_1. The L/U class was formed when S phase cells of a random population were fused with HeLa G_1. The L/L class appeared when DNA synthesis was induced in the unlabeled nucleus of the L/U class of heterokaryons or when both the nuclei in the U/U heterodikaryons entered S phase synchronously. The frequencies of these three classes of heterodikaryons and the labeling index for the mononucleate HeLa G_1 cells were plotted as a function of time for all six fusions but only the data from two fusions, HeLa (random)/HeLa G_1 and WI-38 (random)/HeLa G_1, are presented as examples (Fig. 1).

The rapid decrease in the frequency of the L/U class in HeLa (random)/HeLa G_1 fusion, particularly 2–4 h after fusion indicates that in S/G_1 heterodikaryons DNA synthesis was induced in the G_1 nuclei under the influence of the S phase component (Fig. 1*A*). No such precipitous change in the frequency of the L/U class was noticeable in WI-38/HeLa G_1 fusion (Fig. 1*B*). The decrease in the frequency of L/U class was rather small (8%) and slow, spread over a period of 8 h. A small but gradual increase in the frequency of the L/U class is expected as nuclei of the late G_1 component of the random population residing in the heterokaryons enter S phase. However, this change is too small to mask the precipitous decrease in the frequency of the L/U class that results from the induction of DNA synthesis in the G_1 nuclei of the S/G_1 heterodikaryons. Therefore, these data suggest that the S phase WI-38 cells were relatively poor in their ability to induce DNA synthesis in HeLa G_1 nuclei during the course of the experiment.

We plotted the frequencies of the L/U class (S/G_1) as a

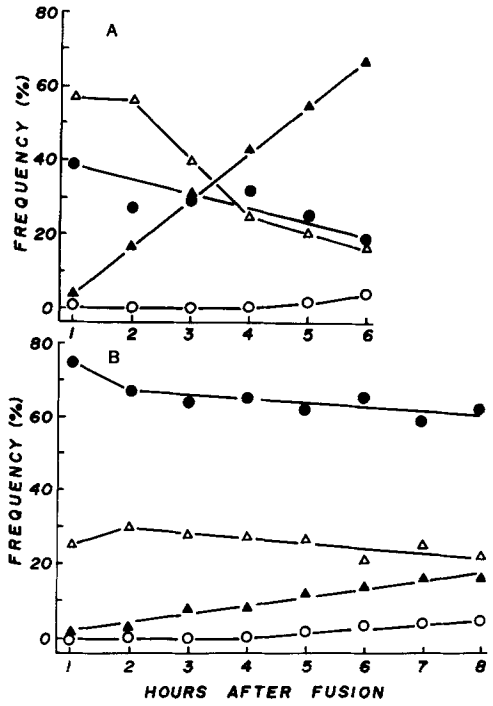

FIGURE 1 Kinetics of induction of DNA synthesis in HeLa G_1 cells after fusion with HeLa or WI-38 cells in exponential growth. WI-38 cells were subcultured regularly at 3–4-d intervals. The labeling index in these cultures ranged between 25 and 30% compared with 35% in random HeLa cells after a 30-min pulse exposure to [^{3}H]-dThd. Vigorously growing subconfluent cultures were trypsinized and plated 2 d before they were used for cell fusion experiments. Random populations of HeLa and WI-38 tagged with small (0.45 μm) latex beads were separately fused with early G_1 phase HeLa tagged with large (0.9 μm) latex beads using UV-inactivated Sendai virus. Latex beads were added to the cultures 18 h before their harvest for cell fusion. After the completion of cell fusion, the fusion mixture was diluted with fresh culture medium containing [^{3}H]-dThd (0.1 μCi/ml; 6.7 Ci/mmol sp act) and plated in a number of 35-mm Lux plastic dishes. Samples were taken at hourly intervals up to 8 h by trypsinizing one of the dishes for each fusion and depositing the cells directly on a clean slide with a cytocentrifuge. The experiments had to be terminated by about 8 h after fusion because HeLa G_1 cells began to enter S phase at this time. The cells were fixed in absolute methanol-acetic acid mixture (3:1), extracted with cold 5% (wt/vol) trichloroacetic acid three times, and then processed for radioautography. The slides were then stained with Giemsa stain and the heterodikaryons and the mononucleate parental types were scored for incorporation of label. Each data point represents an average of three different experiments. About 300 cells were scored for each point. Trinucleate heterokaryons were not scored in this study because it was difficult to determine the number of nuclei contributed by each parent to that cell. (*A*) HeLa (random)/HeLa G_1 fusion. (*B*) WI-38 (random)/HeLa G_1 fusion. ○——○; labeling index of the unfused mononucleate HeLa G_1 cells present in the fusion mixture containing large beads in the cytoplasm. Heterodikaryons with both large and small latex beads in the cytoplasm are represented by: ●——●, both nuclei unlabeled (U/U); ▲——▲, both nuclei labeled (L/L); △——△, one nucleus labeled and the other unlabeled (L/U).

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function of time for all the fusions (data not shown). Initially, there was a slight increase (5–10%) in the L/U class in all the fusions, which subsequently decreased with time (Fig. 1). The initial increase was probably due to the entry of late G_1 nuclei (residing in U/U heterokaryons) into S phase. By 2 h after fusion, all of the cells in S phase had incorporated label; thus, the frequency of the L/U class reached a peak at that time. The subsequent decrease in the frequency of the L/U class was the result of induction of DNA synthesis in HeLa G_1 nuclei by the factors present in the S phase component. To compare the kinetics of induction of DNA synthesis in different fusions, we normalized these data by taking the peak value for each fusion as 100%.

The relative values for the frequency of the L/U class in various fusions were plotted as a function of time (Fig. 2). The faster the induction of DNA synthesis in HeLa G_1, the steeper the slope and hence the higher DSIA in the S phase cells. The times required for a 50% reduction in the frequency of the L/U class were 3.7 h for HeLa, 4 h for SVWI-38, 5.3 h for T98G, and 5.6 h for both AKR-MCA and CHO cells (Fig. 2). In the case of WI-38, the decrease in the L/U class was <20% over a period of 8 h. Therefore, in about 85% of the heterodikaryons of the S/G_1 composition (i.e. L/U class), the S phase cells of WI-38 failed to induce DNA synthesis in HeLa G_1 even up to 8 h after fusion. These data indicate that the DSIA was the highest in S phase cells of HeLa and SVWI-38, while it was the lowest in normal HDF. The DSIA in the chemically transformed AKR-MCA, the spontaneously transformed CHO cells, and T98G human glioblastoma cells was almost identical, being intermediate between the two extremes of normal and HeLa cells.

From the WI-38 (random)/HeLa G_1 fusion, it was not clear whether WI-38 cells in late G_1 could enter S phase after fusion with HeLa G_1. To resolve this question, we fused WI-38 cells partially synchronized in late G_1 with early G_1 HeLa or WI-38

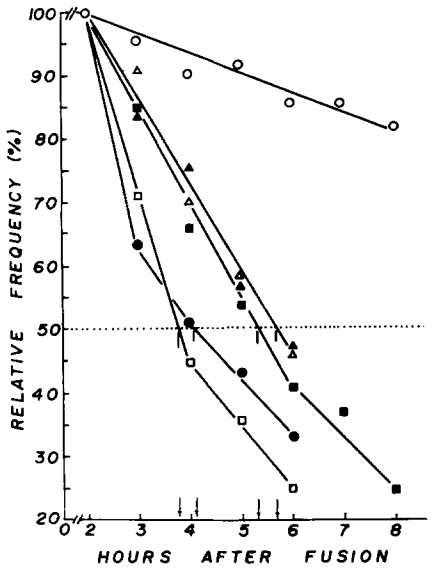

FIGURE 2 Differences in the relative rates of induction of DNA synthesis between the cell types. The relative frequencies of S/G_1 heterodikaryons (L/U class) were plotted as a function of time for all the fusions. The fusions involve HeLa G_1 and a random population of: ○——○, WI-38; □——□, HeLa; ●——●, SVWI-38; △——△, AKR-MCA; ▲——▲, CHO; and ■——■, T98G. The arrows indicate the time required for a 50% decrease in the frequency of the L/U class of heterodikaryons in each fusion. The AKR-MCA and CHO are indicated by the same arrow.

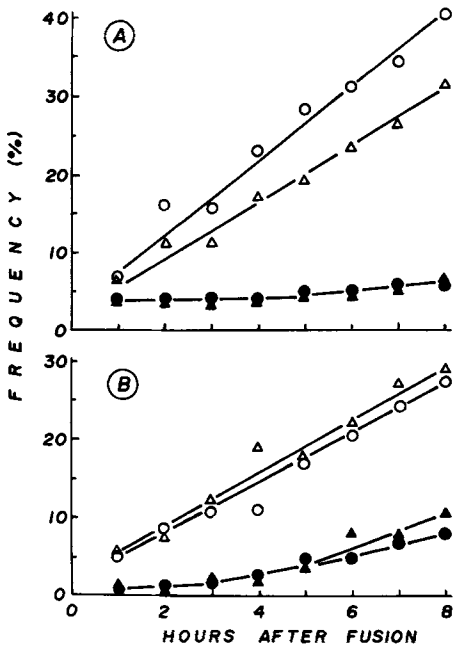

FIGURE 3 Ability of late G_1 WI-38 cells to enter S phase after fusion with quiescent (G_0) WI-38 or HeLa G_1. WI-38 cells in late G_1 phase, tagged with small latex beads, were fused separately with quiescent WI-38 and HeLa G_1 cells that were prelabeled with large latex beads. Quiescent WI-38 cells were obtained by trypsinizing a culture that was in a confluent, stationary phase of growth for 7 d. WI-38 cells were partially synchronized in late G_1 by harvesting cells at 18 h after the subcultivation of a culture that was confluent for 3–4 d. The confluent cultures had a labeling index of <3%. After the completion of fusion, the fusion mixtures were sampled and processed as described under Fig. 1. (*A*) Fusion between WI-38 in late G_1 and WI-38 in G_0. (*B*) Fusion between WI-38 in late G_1 and HeLa G_1. ○——○, labeling index in the unfused mononucleate WI-38 cells in late G_1 carrying small latex beads; ●——●, labeling index in the unfused mononucleate cells of quiescent WI-38 (panel *A*) and HeLa G_1 cells (panel *B*) carrying large latex beads; △——△, frequency of S/G_1 heterodikaryons (with both large and small latex beads) containing a labeled and an unlabeled nucleus (L/U); ▲——▲, frequency of heterodikaryons (containing large and small beads) with both nuclei labeled (L/L).

in G_0 phase (Fig. 3). An almost parallel increase in both the labeling index of mononucleate WI-38 cells and the frequency of the L/U class among the heterodikaryons in both the fusions indicated that in heterokaryons the WI-38 G_0 and HeLa G_1 components had little or no effect on the initiation of DNA synthesis in late G_1 WI-38 cells. However, the WI-38 cells in S phase failed to induce DNA synthesis in G_0 nuclei, as indicated by the absence of any change in the frequency of the L/L class (Fig. 3*A*). In the late G_1 WI-38/HeLa G_1 fusion, the frequency of the L/L class increased to 10% in 8h, which is not significantly different from the labeling index of the mononucleate HeLa G_1 cells (Fig. 3*B*). These results confirmed our earlier observations (on fusion involving random populations) that S phase normal HDF were extremely poor inducers of DNA synthesis in G_1 phase HeLa cells.

Lack of Correlation Between Growth Rate, Cell Size, and DSIA

To find out whether there was any correlation between the DSIA and cell size, we determined the average cell volumes for the different cell types using a multichannel analyzer

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(Channelyzer II, Coulter Electronics, Hialeah, FL) and plotter according to the procedures of Meistrich et al. (13). A comparison of the average cell volumes for the various cell types, their cell cycle times, and their ability to induce DNA synthesis in HeLa G_1 cells is shown in Table I. For example, WI-38 and SVWI-38 were about the same size, but SVWI-38 was an excellent inducer of DNA synthesis while WI-38 was an extremely poor one. AKR-MCA cells were nearly 2.75 times larger in volume than CHO cells, yet their rates of induction of DNA synthesis in HeLa G_1 cells were identical. Similarly, no correlation was found between the cell cycle time (i.e., the growth rate) and the DSIA.

DISCUSSION

In this study we measured the rate of change in the frequencies of L/L, L/U, and U/U binucleate cells formed by fusing synchronized early G_1 HeLa separately with random populations of six different cell types. The rate of decrease in the L/U class is taken as a measure of DNA synthesis-inducing ability of the S phase cells in the random population (Figs. 1 and 2). We realize that new L/U cells are formed as the G_1 component of the random population residing in heterodikaryons traverses through G_1 and enter S phase. This could complicate the interpretation of the results. However, this possibility seems unlikely because the rate at which new L/U cells are formed is too small to mask the precipitous decrease in the L/U class if DNA synthesis were prematurely induced in the unlabeled nucleus under the influence of the S phase factors. The results of this study indicate that the ability of S phase cells to induce DNA synthesis in HeLa G_1 after cell fusion varies with the cell type. Induction of DNA synthesis in G_1 HeLa was rapid by HeLa and virally transformed (SVWI-38) cells, moderate by chemically transformed AKR-MCA and spontaneously transformed CHO and T98G human glioblastoma cells, and very poor by WI-38 (Fig. 2). Cell fusion experiments using CRL 1508, a normal human fetal cell line from American Type Culture Collection, yielded results identical to those from WI-38 (data not shown).

TABLE I

Comparison of Cell Size and Growth Rate of the Various Cell Types and Their Ability to Induce DNA Synthesis in Early G_1 HeLa

Cell type	Approximate cell cycle time	Modal values		DNA synthesis-inducing activity
		Volume	Diameter	
	h *	μm^3	μm	
Random populations				
CHO	13	945	12.6	Good
HeLa	22	1,485	14.16	Excellent
SVWI-38	24	1,575	14.42	Excellent
WI-38	24	1,755	14.96	Poor
T98G	23	2,246	16.2	Good
AKR-MCA	16	2,587	17.0	Good
Synchronized populations				
HeLa mitotic (by N_2O block)		3,307	18.48	
HeLa G_1 (by reversal of N_2O block)		1,642	14.64	
HeLa S (by double thymidine block)		2,812	17.51	

* Based on published data and confirmed by the determination of growth rates for each cell line under our culture conditions.

Table I shows that DNA synthesis-inducing ability is not correlated with either cell size or cell cycle time, i.e., rate of growth. If we assume that the rapid increase in the L/L class, conversely a rapid decrease in the L/U class seen in Fig. 1A, is due to the vigorous proliferating state of HeLa cells, we should expect even a faster decrease in fusions involving CHO cells. CHO cells have a cell cycle time of 13 h compared with 22 h for HeLa. In fact, we found that S phase CHO cells were not so efficient as HeLa cells in inducing DNA synthesis in G_1 phase HeLa cells (Fig. 2).

WI-38 cells partially synchronized in late G_1 were fused with either early G_1 HeLa or G_0 WI-38 to determine their ability to enter S phase after cell fusion (Fig. 3). The rapid increase in the frequency of the L/U class in both fusions (Fig. 3 *A* and *B*) indicates that late G_1 WI-38 are not inhibited by early G_1 HeLa or G_0 WI-38 from entering into S phase. However, the ability of WI-38 S phase cells to induce DNA synthesis in the other nucleus is rather poor as indicated by a very small increase in the L/L class (Figs. 3 *B* and 1 *B*) and a small decrease in the L/U class (Fig. 1 *B*). The differences in the kinetics of the L/L class observed in Figs. 1 *B* and 3 *B* reflect the nature of the WI-38 populations used, i.e., random vs. partially synchronized.

In an earlier study, we had shown that S phase cells have certain factors that can readily induce DNA synthesis in G_1 nuclei in a dose-dependent manner following fusion between S and G_1 phase HeLa cells (23). In similar experiments, de Terra (6) demonstrated that, in the ciliate *Stentor*, DNA synthesis was prematurely induced in G_1 nuclei when transferred into S phase cell, suggesting "the presence of an initiating factor in the cytoplasm during S phase" (20). Using a cell-free assay system, Benbow and Ford (2) observed initiation of DNA synthesis in the isolated nuclei of nondividing liver cells when incubated with the cytoplasmic extracts of rapidly dividing early embryonic cells of *Xenopus laevis*. Using a similar in vitro assay system, Jazwinski et al. (11) showed that the DNA synthesis-inducing activity present in the extracts of proliferating cultured cells is due to one or more nondialyzable and heat-sensitive proteins of molecular weight >50,000.

On the basis of these observations, we propose that the differences in the rate of induction of DNA synthesis exhibited by the various cell types in the present study reflect the variability in the intracellular concentration of DNA synthesis initiators or inducers in these cells. The higher the concentration of the inducers, the more rapid is the rate of induction. Thus, the levels of DNA synthesis inducers were higher in HeLa and virally transformed cells than in normal cells, while those of chemically and spontaneously transformed cells were intermediate between these two extremes. The concentration of these inducers in T98G human glioblastoma cells, which exhibit a G_1 arrest as normal HDF under confluent culture conditions, were lower than in malignant cells but higher than in normal HDF.

These data may explain why HeLa and SV40-transformed HDF in heterokaryons are able to reinitiate DNA synthesis in the nuclei of noncycling senescent and quiescent cells whereas normal HDF are not (16–18, 21, 28–30, 35). The chromatin of quiescent cells is relatively more condensed than that of cycling cells in G_1 phase (1, 4, 10) and hence may require a higher concentration of inducers and longer time for the chromatin to decondense before DNA replication can be initiated (14, 25). The DNA synthesis-inducing activity in HeLa homokaryons is dependent on the ratio of S phase to G_1 nuclei (23). In addition,

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Norwood et al. (17) reported that in heterokaryons involving fusion between HeLa and senescent HDF the ability of HeLa cells to induce DNA synthesis decreased with an increase in the number of senescent nuclei. These observations suggest that the DNA synthesis inducers in S phase HeLa cells can be diluted in heterokaryons to such an extent that they fail to induce DNA synthesis. Therefore, it is predictable that normal HDF with relatively low levels of inducers are unable to induce DNA synthesis in noncycling cells. However, it is rather surprising that S phase WI-38 cells were so poor and so slow in inducing DNA synthesis in HeLa G_1 (Figs. 2 and 3). Probably longer incubation would have led to further induction, but the experiment had to be terminated at 8 h after fusion because of the impending entry of HeLa G_1 cells into S phase. When the HeLa component enters S phase, it would induce DNA synthesis in WI-38 nuclei that are still in G_1 phase at that time.

The transformation of normal cells by viruses or carcinogens is usually associated with a number of phenotypic changes, which include indefinite lifespan in culture, anchorage independence, lower requirement for serum growth factors, absence of G_1 arrest under stationary phase conditions, and higher levels of polyamines. Relative to normal cells, the intracellular concentration of calmodulin has been shown to be elevated twofold to threefold in a variety of virally transformed animal cells (3, 12, 33). These features are collectively known as the transformed phenotype. The results of this study indicate for the first time that the higher intracellular concentration of DNA synthesis inducers is not only one of the pleotropic effects of transformation, but it may also be responsible for some of the features associated with the malignant state such as lack of density-mediated inhibition, low requirement for serum, and ability to induce DNA synthesis in senescent or quiescent cells upon fusion. Maximum DSIA was observed in virally transformed HDF and HeLa. Moreover, microinjection of viral genes can reverse the G_1 arrest of temperature-sensitive cell cycle mutants of 3T3 cells at the nonpermissive temperature (7). Proteins related to the SV40 T antigen have been shown to trigger DNA synthesis in quiescent cells (32). Taken together, these observations tend to support the conclusion that viral transformation results in an increase in the number of copies or the expression of genes that regulate DNA synthesis in general and the inducers of DNA synthesis in particular. This may be a characteristic feature of cellular transformation by DNA tumor viruses since cells transformed by either physical or chemical carcinogens or Rous sarcoma virus are unable to induce DNA synthesis in senescent HDF upon fusion (31). Yanishevsky and Stein (36) earlier speculated that viral transformation may stimulate the production of a new type of inducer. On the basis of the present study, we suggest that virally transformed cells produce more of the same inducer rather than a new type, although our results do not conclusively establish this point.

The present results and those of earlier studies (21, 23, 26) raise the question of whether there are any inhibitors of DNA synthesis per se and, if so, at what point in the cell cycle do they block the cell. Cell fusion studies involving normal HDF with senescent or quiescent cells clearly indicate the presence of certain inhibitory factors in the noncycling cells. The fact that membrane extracts from quiescent cells induce G_1 arrest in a culture of young replicative HDF lends further support to the idea that inhibitors are present in noncycling cells (5, 15, 34). However, in the heterokaryons of normal and senescent or quiescent HDF, these inhibitors have no effect on either the initiation (21, and Fig. 3) or continuation of DNA synthesis (31, 35). Therefore, we can conclude that these inhibitory factors have no direct role in the replication of DNA per se. These inhibitors block the signals required for G_1 traverse rather than the initiation of DNA synthesis. They also differ from the metabolic inhibitors such as hydroxyurea and high concentrations (>2.5 mM) of thymidine and methotrexate which block both the initiation and continuation of DNA synthesis in the treated cells.

We speculate that the inhibitors present in the senescent and quiescent cells block the young replicative nuclei in heterokaryons at a point in early or mid G_1 phase that precedes the expression of genes coding for the synthesis of the inducers of DNA synthesis. Young G_1 cells that have traversed this point at the time of cell fusion with senescent cells can complete their G_1 traverse, synthesize the inducers, and initiate DNA synthesis. In contrast, the G_1 cells that have not yet reached this point at the time of cell fusion will be blocked and will not be able to initiate DNA synthesis even after 2 or 3 d in culture because they are unable to synthesize the inducers. Cells with normal growth regulatory control, such as normal HDF, recognize these inhibitors and stop at a point similar to that of a resting (R) point (19). Transformed cells that have lost their normal regulatory control are not affected by these inhibitors and hence can complete their G_1 traverse and subsequently enter S phase. In this respect the G_1 arrest of young HDF in the young/senescent heterodikaryons may be biochemically analogous to that observed under conditions of serum starvation or deprivation of amino acids.

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