Differential Response of Cycling and Noncycling Cells to Inducers of DNA Synthesis and Mitosis

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ABSTRACT
The objective of this study was to determine whether cells in G0 phase are functionally distinct from those in G1 with regard to their ability to respond to the inducers of DNA synthesis and to retard the cell cycle traverse of the G2 component after fusion. Synchronized populations of HeLa cells in G1 and human diploid fibroblasts in G1 and G0 phases were separately fused using UV-inactivated Sendai virus with HeLa cells prelabeled with [3H]ThdR and synchronized in S or G2 phases. The kinetics of initiation of DNA synthesis in the nuclei of G0 and G1 cells residing in G0/S and G1/G2 dikaryons, respectively, were studied as a function of time after fusion. In the G0/G2 and G1/G2 fusions, the rate of entry into mitosis of the heterophasic binucleate cells was monitored in the presence of Colcemid. The effects of protein synthesis inhibition in the G1 cells, and the UV irradiation of G0 cells before fusion, on the rate of entry of the G2 component into mitosis were also studied. The results of this study indicate that DNA synthesis can be induced in G0 nuclei after fusion between G0- and S-phase cells, but G0 nuclei are much slower than G1 nuclei in responding to the inducers of DNA synthesis because the chromatin of G0 cells is more condensed than it is in G1 cells. A more interesting observation resulting from this study is that G0 cells differ from G1 cells with regard to their effects on the cell cycle progression of G2 cells after fusion. Unlike G1 cells, G0 cells upon fusion with G2 are not able to inhibit the progression of the G2 nucleus into mitosis. This difference between G0 and G1 cells appears to depend on certain factors, probably nonhistone proteins, present in G1 cells but absent in G0 cells. These factors can be induced in G0 cells by UV irradiation and inhibited in G1 cells by cycloheximide treatment.

The cell fusion studies by Rao and Johnson (15) have identified two characteristics that are associated with cells in G1 phase of the cell cycle. They are: (a) inducibility of DNA synthesis by fusion with S-phase cells and (b) the ability of G1 cells to inhibit the progression of G2 cells into mitosis in G1/G2 heterophasic binucleate cells. These observations indicate that G1 cells are deficient in the inducers of DNA synthesis but that G1 chromatin, unlike that of G0, can respond to these inducers and, thus, initiate DNA synthesis when fused with S-phase cells. The inhibition of G2 progression by G1 cells can be explained as follows. It has been well established that chromatin undergoes progressive decondensation during G1 and condensation during G2 (1, 8-12). When G1 cells are fused with those in G0, the decondensation factors of the G1 component seem to neutralize the chromatin condensing factors of the G2 component and, thus, prevent the G2 from entering into mitosis. Do G0 cells have such a capability of inhibiting the progression of a G2 nucleus into mitosis? In a study of heterokaryons formed by fusion of senescent human diploid fibroblasts (HDF) with an immortal cell line (T98G), Stein and Yanishevsky (18) speculated that a noncycling HDF would prevent a T98G nucleus in G2 phase from entering into mitosis. To answer this question, we have decided to compare HDF in G0 phase with those in G1 with regard to two characteristics, i.e., whether DNA synthesis can be induced in G0 cells by fusion with S-phase cells as rapidly as in the case of G1 cells, and whether G0 cells upon fusion with G2 can prevent the latter from entering into mitosis. The results of this study indicate that G0 cells have a 3-4-h lag to respond to the inducers of DNA synthesis and that G0 cells are unable to block the cell cycle progression of a G2 component in G0/G2 heterokaryons.

MATERIALS AND METHODS

Cells and Cell Synchrony

HeLa cells and HDF (strain no. 78-89) were used in this study. HeLa cells were grown as a monolayer culture at 37°C in a humidified 5% CO2 incubator in Eagle's minimal essential medium supplemented with 10% fetal calf serum, sodium pyruvate, glutamine, and antibiotics as previously described (15). These
cells have a generation time of 22 h, and a G1 period of 10.5 h, S phase of 7 h, G2 of 3.5 h, and a mitotic duration of 1 h (14).

HeLa cells were synchronized by the excess ThdR double-block method (14). Synchronized populations of cells in S and G2 periods were obtained by collecting cells at 1 and 7 h, respectively, after the reversal of the second ThdR block. A pulse labeling with [3H]ThdR revealed a labeling index of 96% in S-phase cells and a labeling index of 5% in G2 population. The mitotic index was <2% in both S and G2 populations. Early G1 population was obtained by collecting the cells at 2 h after the release of a N20 block after the reversal of a single excess ThdR block (13). The G1 population had a mitotic index of 5% and a 0% labeling index.

The HDF strain we used was kindly supplied by Dr. Thomas Norwood of the University of Washington (Seattle, Wash.). HDF were grown as monolayers in McCoy's modified 5A medium supplemented with 20% fetal calf serum, glutamine, and antibiotics. HDF were in their 15-18th passage during the period of these experiments. HDF in Go were obtained by harvesting at 7 d after cells had reached confluence. To obtain HDF in G1 phase, the cells were held in confluence for 7 d and then trypsinized and replated at 25% confluence. At 18 h after plating, cells were harvested for fusion. The labeling index was <2% in G1 and Go populations.

Cycloheximide Treatment

Mitotic HeLa cells were obtained by selective detachment from dishes that were exposed to N20 (80 pounds per square inch) for 10 h. By this method, we can obtain large amounts of mitotic cells of high (98%) purity. These mitotic cells were plated in new dishes in a medium containing cycloheximide (25 μg/ml) and incubated for 8 h, i.e., until the time of fusion. At this concentration of cycloheximide, ~95% of protein synthesis is inhibited in mammalian cells (4). Inhibition of protein synthesis by cycloheximide had no effect on the completion of mitosis and cytokinesis. These cells may be considered to be blocked in early G1.

UV Treatment

HDF in Go were trypsinized and plated into three 60-mm culture dishes in 2 ml of medium. One dish (with the lid off) was exposed to UV for 60 s (21.3 J/m²/s) from a Sylvania germicidal lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.) and another dish for 30 s. The third dish, not exposed to UV light, served as a control. Immediately after they were irradiated, the cells were fused with HeLa cells in G1 phase.

Cell Fusion

The procedure we used for UV-inactivated Sendai virus has been previously described (15). To study the regulation of DNA synthesis, we performed three different fusions. They were: (a) HeLa S'/HeLa Gt, (b) HeLa S'/HDF Gt, and (c) HeLa S'/HDF Go. (The asterisk indicates the cell population that was prelabeled with [3H]ThdR during the synchronization procedures.) Immediately after fusion between a prelabeled and an unlabeled population, each of the fusion mixtures was resuspended in regular medium. About 1 ml of this cell suspension was placed on one of the dishes with a cytomegalovirus (Shandon-Elliott Co., London, England). To the remaining cell suspension, Coloemid (0.05 μg/ml) and Colcemid (0.05 μg/ml) were added and plating was immediately done in a number of 35-mm culture dishes. Cell samples were taken at hourly intervals by trypsinizing one of the dishes. The trypsinized cells were deposited on slides as described above, fixed in 3:1 methanol-glacial acetic acid mixture, processed for autoradiography, stained with Giemsa's, and scored for the frequency of labeled nuclei among mono-, bi-, and trinucleate cells. About 500 cells were scored for each time point. The data presented are the averages of the three different experiments.

The procedures for estimating the rate of induction of DNA synthesis in G0 nuclei after fusion between G0 and S-phase cells have been previously described (15). Briefly, they are as follows. Before fusion, the cells of each population were mononucleate and either labeled (L) or unlabeled (U). After fusion, we scored mononucleate Go cells remaining <2% throughout this experiment. With the slower response of G0 nuclei to the S-phase inducers caused by the absence of any inducer molecules in G0 cells relative to those of G1, one would expect a rapid induction of DNA synthesis in G0 nuclei by increasing S-phase component in the fused cells. To find out whether increasing the ratio of S:Go would alter the kinetics of initiation of DNA synthesis in G0 nuclei, we scored trinucleate cells containing 1 S.2 Go or 2 S:1 G0 nuclei for labeling index (Fig. 2). These data indicate that increasing the ratio of S:G0 by a factor of two advanced the entry of the G0 nuclei into S phase by only 0.5 h. When this ratio was reversed, i.e., 1 S:2 G0, the entry of both the G0

![Figure 1](https://example.com/figure1.png)
nuclei into S phase was delayed by ~30 min. However, some
asynchrony was observed with regard to the initiation of DNA
synthesis in the G0 nuclei of the trinucleate (1 S:2 G0) cells. In
~25% of the cases, one of G0 nuclei incorporated [3H]Tdr whereas the other one did not. Within the next 30 min, the
second nucleus also became labeled.

Regulation of Mitosis in the Fused Cells

HeLa cells in G2 period (prelabeled with [3H]Tdr) were
fused separately with G1, HeLa and G1 or G0 population of
HDF, and the rate of mitotic accumulation in the presence of
Colcemid was determined for the mono- and binucleate cells.
The kinetics of mitotic accumulation in different types of
binucleate cells were compared (Fig. 3). The mono- and binu-
clide G2 cells were the first to enter mitosis and reach a MI of
50% by 5.5 h after fusion. However, the G1/G2 heterodiakary-
ons were delayed significantly in their entry into mitosis and
their MI remained <2% during the course of this experiment.

In contrast, the rate of entry into mitosis of G0/G2 hetero-
dikaryons was intermediate between those of G2/G2 and G1/
G2 binucleate cells. In this case, the G2 component entered
normal mitosis whereas the G0 nuclei underwent premature
chromosome condensation. The G0/G2 binucleate cells had a
MI of 46% at 8 h as compared with the 70% MI of the G2/G2
binucleate cells. This indicates that G0/G2 binucleate cells are
some what slower than the G2/G2 binucleate cells in their rate
of entry into mitosis. These findings demonstrate a functional
difference between the noncycling G0 and cycling G1 cells, i.e.,
the G1 component inhibits the progression of the G2 component
in a fused cell from entering into mitosis, whereas the G0
component lacks this ability. However, when HeLa cells ar-
ested in G1 phase (by treating mitotic cells with cycloheximide)
were fused with G2 cells, ~20% of G1/G2 binucleate cells
entered mitosis within 6 h as compared with 2% or 3% in the
control (Fig. 4).

Because UV irradiation of mammalian cells is known to
induce decondensation of chromatin (6, 17, 19), we wanted to
investigate the effects of UV irradiation of G0 cells and their
subsequent fusion with HeLa G2 cells on the rate of entry of
G0/G2 binucleate cells into mitosis. In these experiments, we
have observed that the exposure of G0 cells to UV light before
fusion retarded the progression of the G2 component into
mitosis (Fig. 4). The higher the dose of UV irradiation, the
slower is the rate of entry of G0/G2 binucleate cells into mitosis.

DISCUSSION

The results of this study indicate that DNA synthesis can be
induced in G0 nuclei after fusion between G0- and S-phase
cells, but that G0 nuclei are much slower than G1 nuclei in

FIGURE 2 Effect of S:G0 ratio in the trinucleate cells on the kinetics
of labeling of G0 nuclei. HDF in G0 were fused with prelabeled
HeLa cells in S phase. The LI of G0 nuclei residing in trinucleate
cells consisting of 1 S:2 G0 or 2 S:1 G0 were compared with those in
the binucleate (1 S:1 G0) cells. (O), G0 nuclei in binucleate (1 S:1
G0) cells; (1), G0 nuclei in trinucleate (2 S:1 G0) cells; (A), G0 nuclei
in trinucleate (1 S:2 G0) cells. The arrows indicate the time required
for each class of cells to achieve a 50% LI. The dotted line indicates
a 50% level.

FIGURE 3 The rate of mitotic accumulation in G0/G2 and G1/G2
fusions. HeLa cells in G1 and HDF in G1 and G0 phases were
separately fused with prelabeled HeLa cells synchronized in G2.
The MI were scored for the mono- and binucleate populations and
plotted as a function of time. Data involving HDF- G1/HeLa G2
fusion are not presented because they were identical to the data
from HeLa G1/HeLa G2 fusion. (O), Homophasic binucleate cells,
G2/G2; (1), heterophasic binucleate cells, G0/G2; (A), heterophasic
binucleate cells, G1/G2. The MI for mononucleate G0, G1 cells, and
the homophasic binucleate cells, i.e., G1/G1; and G0/G0 were <2%
and, hence, are not included in the figure.

FIGURE 4 Effects of the inhibition of protein synthesis in G1 and
UV irradiation of G0 cells before fusion on the cell cycle traverse of
the G2 component in G0/G2 and G1/G2 dikaryons. HeLa cells
arrested in G1 by treating mitotic cells with cycloheximide were
fused with prelabeled HeLa G2. HDF in G0 were UV irradiated for
60 s and then fused with HeLa G2. The rate of mitotic accumula-
tion of the binucleate cells in the presence of Colcemid was deter-
mined as a function of time. (O), HeLa G0/HDF - G0 untreated;
(1), HeLa G0/HDF - G0 UV irradiated for 60 s; (A), HeLa G0/HeLa
G1; arrested by cycloheximide treatment; (1), HeLa G0/HeLa
G1, untreated.
responding to the inducers of DNA synthesis (Fig. 1). After fusion between a "quiescent" and an S-phase population of 3T3 cells, Brooks (3) observed that the rate of induction of DNA synthesis in the quiescent (G₀) nuclei residing in the heterophasic (G₀/S) binucleate cells was remarkably slow. The G₀ nuclei became labeled only in 10% of the G₀/S binucleate cells at 4 h after fusion. This had risen to 51% at 8 h and to 76% at 12 h (3). Even though Brooks has referred to these quiescent cells as G₁ cells, in light of this study, it would appear that he was actually dealing with G₀ cells. His results could be explained by assuming that the quiescent cells were in a state of deeper G₀ and, hence, would take a longer time to respond to the inducers of DNA synthesis. The slow response of the G₀ nuclei observed by us and by Brooks could be caused by one of the following reasons. (a) Because the cycling G₁ cells are progressing towards S phase, they are likely to contain relatively more molecules of the inducers of DNA synthesis than are the noncycling G₀ cells. This difference could result in the early onset of DNA synthesis in G₁ nuclei after fusion with S-phase cells. (b) The conformational pattern of chromatin of G₀ cells is different from that of G₁ chromatin. It is evident from the literature that the chromatin of G₀ cells is more condensed than it is in G₁ cells (2, 7). Because the G₀ chromatin is more condensed, it takes ~2-3 h after fusion with S-phase cells to become decondensed and be able to initiate DNA synthesis (Fig. 1). In light of these data, the second possibility appears to be more likely than the first (Fig. 2). If the absence of inducer molecules in G₀ cells is the cause of delayed initiation of DNA synthesis in G₀ nuclei of G₀/S binucleate cells, one would expect a rather rapid initiation by doubling the ratio of S-phase components to the G₀ component. This expectation is based on the model for nonconcentration dependent cooperative initiation of DNA synthesis proposed by Fournier and Pardee (5) and later confirmed by Rao et al. (16). A 50% LI in 1 G₀,2 S trinucleate cells was reached at 4.25 h compared with 4.45 h in the case of 1 G₀,1 S binucleate cells (Fig. 2). Therefore, doubling the number of inducer molecules, as in the case of 2:1 G₀ trinucleate cells, did not result in a significant advancement in the rate of entry of the G₀ nucleus into S phase. Reversing this ratio to 2 G₀,1 S caused only a very small delay (<30 min) in the entry of these cells into S phase. From the foregoing discussion, it appears that differences in the conformation of chromatin in G₀ and G₁ cells may be a cause of their differential response to inducers of DNA synthesis.

A more interesting observation resulting from this study is that G₀ cells differ from G₁ cells with regard to their effects on the cell cycle progression of G₂ cells after fusion. The fusion between G₁ and G₂ cells inhibited the G₀ component's entry into mitosis in G₀/G₂ dikaryons (Fig. 3). In contrast, in G₀/G₂ dikaryons, the G₀ component caused only a slight delay in the entry of the G₂ nucleus into mitosis and the consequent premature chromosome condensation of the G₀ nucleus (Fig. 3). This difference between G₀ and G₁ appears to depend on certain factors, perhaps nonhistone proteins, present in G₁ cells but absent in G₀ cells. In earlier studies (16), we have shown that there is a progressive decondensation of chromatin during G₁ that is associated with accumulation of inducers of DNA synthesis. The proteins synthesized during G₁ period may be responsible for the decondensation of chromatin, whereas those synthesized during G₂ may be responsible for chromatin condensation. Hence, it is possible that, in a binucleate cell formed by the fusion of G₁ and G₂ cells, the condensation factors of the G₂ component are neutralized by the decondensation factors of the G₁ component and, thus, the cell cycle progression of G₂ nucleus is delayed until G₁ nucleus completes DNA synthesis. This suggestion is further supported by the fact that G₁ cells, in which protein synthesis was inhibited, were not so effective in blocking the progression of the G₂ component as the control G₁ cells (Fig. 4).

In this study, we have also demonstrated that the influence of the G₀ component on the rate of entry into mitosis of G₀/G₂ dikaryons can be modified by UV irradiation of G₀ cells before fusion (Fig. 4). The fusion of UV-irradiated G₀ HDF with HeLa cells in G₂ resulted in a significant retardation in the rate of entry of G₀/G₂ dikaryons into mitosis. This appeared to be dose dependent (the data for a 30-s exposure to UV are not presented). UV irradiation is known to induce substantial unscheduled DNA synthesis in G₁ and G₂ nuclei, which reflects repair replication of UV-damaged DNA. We have also shown that G₀ chromosomes of cells irradiated with UV in G₁ phase are elongated and attenuated and appear similar to the prematurely condensed chromosomes of S-phase cells. Further studies by Schor et al. (17) have revealed a close correlation between the degree of chromosome decondensation and the amount of unscheduled DNA synthesis induced by UV irradiation during G₁ and mitosis. UV-irradiated mouse fibroblasts were shown to incorporate more acridine orange in their nuclei than the unirradiated controls (6). The amount of an intercalating dye, such as acridine orange, bound to DNA has been shown to be directly proportional to the degree of chromatin decondensation (12). Therefore, a significant change in the UV-irradiated G₀ cells would be the decondensation of chromatin and the activation of the DNA repair synthesis. In light of these facts, we suggest that the factors induced by UV irradiation that cause chromatin decondensation may counteract the condensation factors present in the G₂ component and, thus, delay the entry of G₀/G₂ dikaryons into mitosis. We have made a similar suggestion earlier to explain the inhibition of progression of the G₀ component into mitosis in G₁/G₂ or S/G₂ binucleate cells (15). However, the exact molecular basis for this phenomenon remains to be elucidated.

In conclusion, this study shows that cells in G₀ phase are functionally distinct from those in G₁ phase with regard to their ability to respond to inducers of DNA synthesis, and to inhibit the progression of G₂ nuclei into mitosis.

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Note Added in Proof: The report by W. E. Mercer and R. A. Schlegel (1980, Exp. Cell Res. 128:431-438), published while our paper was in press, indicates that there is a lag in the initiation of DNA synthesis in quiescent (G₀) nuclei after fusion between quiescent and S-phase 3T3 cells. These results are in complete agreement with those of ours.

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