Effect of Cycloheximide on the Reactivation of Chick Erythrocytes in Heterokaryon Formation with BHK Cells

Yoshihiro Tsutsui

Department of Pathology, Nagoya University, School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan

ABSTRACT. Induction of DNA synthesis in chick erythrocytes after fusion with BHK cells was compared in the early, middle and late S phase. In heterokaryons fused with early S phase cells, more than 55 % showed the induction of DNA synthesis in the chick nuclei even in the presence of cycloheximide (1 μ g/ml). However, in heterokaryons fused with late S phase cells, less than 20 % showed the induction of DNA synthesis in the chick nuclei in the presence of the same amount of cycloheximide. These results suggest that the induction of DNA synthesis in chick nuclei in the early S phase does not depend on protein synthesis because of the accumulation of inducers and because in the late S phase protein synthesis is necessary for the induction of DNA synthesis in chick nuclei.

Chick erythrocytes can be reactivated when fused with growing mammalian cells (4, 9). In a previous study, we used ts mutants which arrest at a non-permissive temperature in G1 to show that chick nuclei in heterokaryons are reactivated when host cells are in the S phase or are capable of reaching this stage (14). Only cells, or cytoplasm from S phase cells, had all the necessary information to reactivate DNA synthesis in chick nuclei after fusion (14, 6). Thus, reactivation of the chick erythrocyte depends on the presence of certain factors produced by S phase cells.

However, it is commonly understood that in mammalian cells preparations for the replication of DNA are generally made during the G1 period (8). Inhibition of protein synthesis in G1 blocks the progression of cells into the S phase, which suggests that these initiators are probably proteins (2). It is not clear whether these initiators are the same as the inducers of DNA synthesis present in S phase cells.

We here describe the effect of cycloheximide on the induction of DNA synthesis in chick erythrocytes in heterokaryons fused with BHK cells that had been synchronized in early, middle and late S phase.

BHK 21/13 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco) plus 10% calf serum and 100 U/ml penicilin and 50 μ g/ml streptomycin. BHK cells were plated on coverslips in 10 cm Petri dishes (Falcon) for 3 days with a medium containing 10% serum. The cells were washed with DMEM after which they were incubated in the DMEM with 0.25% serum for 48 h. Then, the cells were stimulated with a 10% serum-containing medium containing 1 mM hydroxyurea for

Abbreviations: BHK, baby hamster kidney; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate buffered saline.

15 h. The cells arrested at the G1/S boundary were released into the S phase by washing them several times then incubating them in fresh medium containing 10% serum. To check the synchronization of the BHK cells, we labeled the cells with 0.5 $\mu\text{Ci/ml}$ of [^3H] thymidine for 1 h prior to the washing and fixation of the coverslips. Three hours after their release from hydroxyurea, 72% of the cells were labeled by autoradiography, and 6 and 9 h after their release 85% and 66% of the cells were labeled.

To examine the effect of cycloheximide on protein and DNA syntheses in BHK cells, 2 h after their release from hydroxyurea, we incubated the cells with various concentrations of cycloheximide and 2 $\mu\text{Ci/ml}$ [^3H] leucine or 1 $\mu\text{Ci/ml}$ [^3H] thymidine for 1 h in a medium containing a concentration of leucine that was 1/5 that of the complete medium. At the end of the pulse, cells were washed with PBS then lysed in lysate buffer; radioactivity was counted in a Beckman liquid scintillation counter after the procedure described by Talavera and Basilico (13).

When protein synthesis in cells without cycloheximide treatment was taken as 100%, protein synthesis was reduced to 35%, 17% and 8% when the concentration of cycloheximide was increased to 0.2 $\mu\text{g/ml}$, 1.0 $\mu\text{g/ml}$ and 5.0 $\mu\text{g/ml}$. As to the effect of cycloheximide on DNA synthesis in BHK cells, the incorporation of [^3H] thymidine was reduced to 30% within 1 h in the presence of 0.2 $\mu\text{g/ml}$ of cycloheximide and reduced to 20% when 1.0 $\mu\text{g/ml}$ was added.

Cell fusion between the BHK cells and chick erythrocytes was carried out in monolayers in the presence of UV-inactivated Sendai virus as described previously (14). After fusion the cells were washed gently then incubated in fresh medium with 10% serum plus various concentrations of cycloheximide. The heterokaryons were exposed continuously to 0.2 $\mu\text{Ci/ml}$ of [^3H] thymidine, after cell fusion for 20 h. At least 200 heterokaryons were counted for each experimental measurement.

In heterokaryons with early S phase cells, the induction of DNA synthesis in chick nuclei was higher than in heterokaryons with middle and late S phase cells (Fig. 1A). In the absence of cycloheximide, the result was close to the results of Johnson and Mullinger which showed that the induction potential of late S phase HeLa cells is somewhat lower than that of early and middle S phase cells (5). In the presence of cycloheximide (1 $\mu\text{g/ml}$), the induction of DNA synthesis in the chick nuclei occurred in more than 55% of the heterokaryons combined with early S phase cells. However, the induction of DNA synthesis in the chick nuclei was remarkably reduced, to less than 22% in the presence of the same amount of cycloheximide in the heterokaryons combined with late S phase cells (Fig. 1A). As shown in Fig. 1B, the percentage of heterokaryons with labeled BHK nuclei but not labeled chick nuclei was increased remarkably to more than 60% in the presence of cycloheximide when late S phase cells were used.

Inhibition of protein synthesis decreased the rate of DNA synthesis. Reduction of DNA synthesis occurs rapidly after the inhibition of protein synthesis (12, 15). However, the reduction in DNA synthesis is not complete for at least one round of DNA replication in the absence of protein synthesis (11). In heterokaryons fused with early S phase cells, the induction of DNA synthesis in chick nuclei occurred even in the presence of a relatively high concentration of cycloheximide (5 $\mu\text{g/ml}$) (2). In contrast, in heterokaryons fused with late S phase cells, the induction of DNA synthesis in chick nuclei decreased in the presence of cycloheximide. This seems to indicate that early S phase cells have large amounts of inducers for the induction of

Fig. 1. BHK cells were synchronized as described in the text, then 3, 6, 9 and 12 h after their release from hydroxyurea, they were treated with 1 µg/ml of cycloheximide which was added to the medium. After fusion, the heterokaryons were incubated in a medium containing 0, 0.2, 0.6, 1.0 and 5.0 µg/ml of cycloheximide, plus 0.2 µCi/ml of [³H]thymidine but no serum. A, % of heterokaryons in which both BHK nuclei and chick nuclei were labeled. B, % heterokaryons in which BHK nuclei were labeled, but chick nuclei were not. Autoradiography were carried out as described by Baserga and Malamud (1).

DNA synthesis in chick nuclei, and that the induction of DNA synthesis is not completely dependent on protein synthesis. Because of the marked reduction of the induction of DNA synthesis in chick nuclei in the presence of cycloheximide in the late S phase, translation from mRNA to inducers for the induction of DNA synthesis in chick nuclei may be blocked by cycloheximide, although the mRNAs, themselves, are maintained at certain levels. However, the different effects of cycloheximide on the induction potential of DNA synthesis in chick nuclei between the early and late S phase heterokaryons may mean that the mechanism of induction for DNA synthesis in the late S phase may differ completely from that in the early S phase.

In multinucleate organisms, all the nuclei within a cell generally enter the S phase synchronously (3). This synchrony in the initiation of DNA synthesis has been demonstrated in G1 nuclei after fusion between G1 and S phase cells of the same and different species (5, 7, 8). Our present results, however, have shown that in the presence of cycloheximide, the induction of DNA synthesis in chick nuclei decreased remarkably in heterokaryons fused with late S phase cells, although the BHK nuclei were labeled with [³H]thymidine (see Fig. 1B). This kind of asynchrony in the induction of DNA synthesis also has been observed in chick erythrocytes fused with S phase tsAF8 cells which had been stimulated from quiescence to induce DNA synthesis by adenovirus 2 at a non-permissive temperature (10). The Asynchrony of DNA synthesis by cycloheximide in nuclei fused with late S phase cells may be one model for the study of the induction of DNA synthesis.

Acknowledgments. We thank Dr. Baserga, Temple University, Philadelphia, for his helpful advice and Dr. Munemitsu Hoshino, Nagoya University, for his constant encouragement.

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(Received for publication, February 29, 1980)