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NOTE

Adenovirus Type 5 Induces Progression of Quiescent Rat Cells into S Phase Without Polyamine Accumulation

B. F. CHEETHAM,[1]*† D. C. SHAW,[2] AND A. J. D. BELLETT[1]

Departments of Microbiology[1] and Physical Biochemistry,[2] John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia

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Adenovirus type 5 induces cellular DNA synthesis and thymidine kinase in quiescent rat cells but does not induce ornithine decarboxylase. We now show that unlike serum, adenovirus type 5 fails to induce *S*-adenosylmethionine decarboxylase or polyamine accumulation. The inhibition by methylglyoxal bis(guanylhydrazone) of the induction of thymidine kinase by adenovirus type 5 is probably unrelated to its effects on polyamine biosynthesis. Thus, induction of cellular thymidine kinase and DNA replication by adenovirus type 5 is uncoupled from polyamine accumulation.

Rat embryo fibroblasts cultured in medium with 0.2% serum become quiescent and arrested in the G1 phase of the cell cycle. The addition of fresh medium containing 10% serum initiates a series of biochemical events which lead to DNA synthesis and cell division. Infection of quiescent rat cells by adenovirus type 5 (Ad5) also induces cellular DNA synthesis (3) and thymidine kinase (3a), an enzyme whose activity increases as the cells enter S phase (16).

The induction of DNA synthesis is preceded by the induction of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, two key enzymes of polyamine biosynthesis, in a wide variety of systems (17). Further, inhibitors of these two enzymes inhibit subsequent DNA synthesis under several different conditions (2, 7–9, 18). It thus appears that polyamine accumulation in G1 is necessary for the entry of quiescent cells into S phase.

Ad5, however, differs from serum in that it induces cellular thymidine kinase and DNA synthesis without inducing ornithine decarboxylase (3a). Further, the induction of thymidine kinase by Ad5, unlike its induction by serum, is not inhibited by α-methylornithine, an inhibitor of ornithine decarboxylase (3a). Methylglyoxal bis(guanylhydrazone) (MGBG), an inhibitor of *S*-adenosylmethionine decarboxylase, inhibits the induction of thymidine kinase by both serum and Ad5. To further investigate the requirement for polyamine biosynthesis in the Ad5-induced and serum-induced entry of quiescent cells into S phase, we measured *S*-adenosylmethionine decarboxylase activity and polyamine levels in rat cells after stimulation by serum or infection with Ad5.

Rat embryo fibroblasts were mock infected, infected with Ad5, or changed to fresh medium containing 10% fetal calf serum, and the *S*-adenosylmethionine decarboxylase activity was measured at 2-h intervals over the next 22 h.

To confirm that the cells were successfully infected with Ad5, thymidine kinase activity was measured in mock-infected and Ad5-infected cells at 48 h after infection. Early viral antigen expression was also measured by staining with P antiserum at this time (5).

S-Adenosylmethionine decarboxylase activity increased 4 h after stimulation by serum and reached a maximum level about threefold higher than that in the mock-infected cells at 6 h (Fig. 1). The activity of the enzyme then declined, but it increased again after 16 h. In Ad5-infected cells, there was no increase in *S*-adenosylmethionine decarboxylase activity above the level in mock-infected cells (Fig. 1). The Ad5-infected cells did show increased thymidine kinase activity, and 30% of the cells were positive for expression of early antigens as determined by P antiserum staining (data not shown).

Hence, adenovirus infection induces cellular thymidine kinase without inducing two key en-

† Present address: Department of Biological Sciences, University of California Santa Barbara, Santa Barbara, CA 93106.

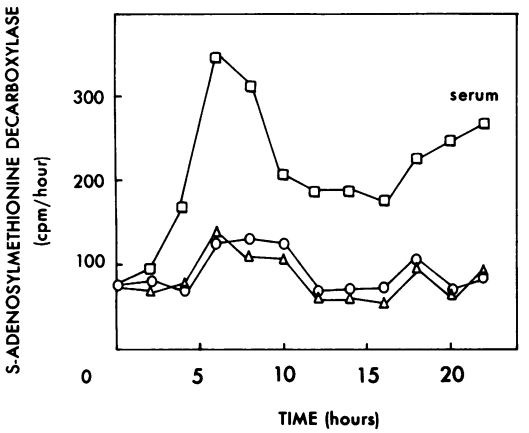

FIG. 1. Induction of *S*-adenosylmethionine decarboxylase by serum, but not by Ad5, in low-serum-arrested rat embryo fibroblasts. Methods for the growth of cells and virus, arrest of cells in 0.2% serum, infection of cells, and preparation of cell extracts have been described previously (3a). At time zero, cells were mock infected (○), infected with Ad5 (△), or changed to fresh medium with 10% fetal calf serum (□). At 2-h intervals, cell extracts were prepared, and *S*-adenosylmethionine decarboxylase activity was measured by the release of $^{14}CO_2$ from *S*-adenosyl-L-[carboxyl-^{14}C]methionine. The assay was based on that of Heby et al. (6). Detailed methods will be published elsewhere (B. F. Cheetham and A. J. D. Bellett, submitted for publication).

zymes in polyamine biosynthesis, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase. Polyamine levels were next measured 36 h after the infection with Ad5 or the stimulation by serum of low-serum-arrested rat embryo fibroblasts. The effects of α-methylornithine, an inhibitor of ornithine decarboxylase (1), and MGBG, an inhibitor of *S*-adenosylmethionine decarboxylase (19), were also determined.

The putrescine and spermidine content was much greater in serum-stimulated cells than in mock-infected cells (Table 1). However, there was no increase in spermine content after serum stimulation. In Ad5-infected cells, the levels of putrescine, spermidine, and spermine were lower than in the mock-infected cells. Hence, serum, but not Ad5, induces polyamine accumulation in low-serum-arrested rat embryo fibroblasts.

α-Methylornithine treatment greatly reduced the levels of putrescine and spermidine in the cells stimulated by serum (Table 1). Putrescine and spermidine levels were also reduced in the Ad5-infected cells. However, spermine levels did not decrease under either condition. The reason for this is not known.

In serum-stimulated cells, MGBG treatment resulted in a large increase in putrescine and a decrease in spermidine and spermine content (Table 1). The decrease in spermidine and spermine content was probably sufficient to account for the inhibition of the induction of thymidine kinase. In the cells infected by Ad5, MGBG caused a large increase in putrescine content (Table 1). However, spermidine content decreased by only about 35%, and spermine content was unchanged. Since the decrease in spermidine content was no greater than that in the α-methylornithine-treated infected cells, and since α-methylornithine does not inhibit the induction of thymidine kinase by Ad5 (3a), it is unlikely that the inhibition by MGBG of the induction of

TABLE 1. Reduction of polyamine levels in rat embryo fibroblasts by MGBG and α-methylornithine[a]

Treatment of cells	Thymidine kinase activity (nmol/mg per 30 min)	Polyamine (nmol per dish)		
		Putrescine	Spermidine	Spermine
Mock infected	0.02	0.06	0.76	2.32
Ad5	0.08	0.04	0.60	0.80
Ad5 + α-methylornithine	0.08	<0.02	0.42	1.20
Ad5 + MGBG	0.02	0.56	0.40	0.86
Serum	0.26	0.14	2.96	1.70
Serum + α-methylornithine	0.10	<0.02	0.32	2.16
Serum + MGBG	0.06	2.32	0.64	0.62

[a] At time zero, low-serum-arrested rat embryo fibroblasts (approximately 4×10^5 cells per dish) were mock infected, infected with Ad5, or changed to fresh medium containing 10% fetal calf serum. α-Methylornithine or MGBG was added at the same time as serum, or at the end of the virus adsorption period. Thymidine kinase activity was measured at 36 h (serum) or 48 h (Ad5) by methods described previously (3a). Putrescine, spermidine, and spermine were separated by ion exchange chromatography and estimated by using *o*-phthaldehyde by a modification of the method of Marton and Lee (10). Detailed methods will be published elsewhere (A. J. D. Bellett, L. K. Waldron-Stevens, D. C. Shaw, and B. F. Cheetham, submitted for publication).

TABLE 2. Failure of MGBG to prevent viral early antigen expression in rat cells[a]

Treatment of cells	Thymidine kinase activity (nmol/mg per 30 min)	P antigen-positive cells (% of total)
Mock infected	0.06	0
Ad5	0.52	49
Ad5 + MGBG	0.04	48

[a] Low-serum-arrested cells grown in dishes containing cover slips were mock infected or infected with Ad5. MGBG (10 μM) was added at the end of the adsorption period. Cover slips were fixed at 48 h and stained with P antiserum (5). Thymidine kinase activity was measured at 48 h.

thymidine kinase by Ad5 is due to spermidine depletion. The inhibition by MGBG of the induction of thymidine kinase by Ad5 and serum can be prevented by adding spermine and spermidine (3a). However, this does not rule out the possibility that the effects of MGBG are not due to its effects on polyamine biosynthesis.

In addition to its effect on polyamine accumulation, MGBG has been reported to cause damage to mitochondria (4, 11–13). Also, MGBG is actively taken up by cells, and spermidine and spermine interfere with the uptake of the drug and reduce its intracellular level to below that required to inhibit growth (15). Hence, the inhibition by MGBG of the induction of thymidine kinase could be due to polyamine depletion or to side effects of the drug, such as mitochondrial damage, or to accumulation of *S*-adenosylmethionine or putrescine, since spermidine and spermine would, in either case, prevent this inhibition.

We have previously shown that the induction of thymidine kinase by Ad5 requires an early gene product other than the DNA-binding protein or the gene N product. MGBG could prevent the induction of thymidine kinase by Ad5 by interfering with the expression of adenovirus early genes. We tested this possibility by staining drug-treated infected cells with P antiserum, which reacts with adenovirus early proteins (5). No difference in the number of cells scored as positive for P antigen was obtained between MGBG-treated and untreated cells, despite a large inhibition by MGBG of thymidine kinase induction in this experiment (Table 2). Hence, it is unlikely that MGBG inhibits thymidine kinase induction in adenovirus-infected cells by preventing early gene expression.

We have thus demonstrated that the adenovirus-induced entry of cells into S phase is independent of polyamine accumulation. Adenoviruses also induce cellular DNA synthesis without RNA accumulation, an event which normally occurs in G1 (14). These results are consistent with the proposal that adenoviruses induce cellular DNA synthesis without some of the early events in the G1-to-S-phase progression. An adenovirus early protein may act on a control point late in the G1 phase to induce an abnormal cell cycle. Further biochemical studies on the adenovirus-induced and serum-induced G1-to-S-phase transitions should lead to a greater understanding of the mechanism by which these two agents induce cellular DNA synthesis. In addition, the adenovirus system may perhaps be used to investigate the consequences of S-phase entry without prior accumulation of polyamines, and so it may further delineate the roles of polyamines in cell cycle progression.

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