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CALMODULIN AND CELL PROLIFERATION

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The calmodulin content of synchronized Chinese hamster ovary (CHO-K_1) cells was determined at each phase of the cell cycle. The calmodulin content was minimum in the G_1 phase, increased after the cells entered S phase and reached the maximum level at the late G_2 or early M phase. When 30 μM of W-7 (calmodulin antagonist) was added at the S phase, the cell cycle was blocked at the late G_2 or early M phase. The addition of W-7 also prevented the morphological changes caused by cholera toxin. These results suggest that calmodulin plays an important role in the phases through S to M, possibly in the initiation of DNA synthesis and in the mitosis.

INTRODUCTION

Calcium ion has been implicated in the regulation or modulation of various cell processes such as cell division (1), initiation of DNA synthesis (2, 3), cell adhesion (4), motility (5), exocytotic secretion in endocrine and exocrine glants (6, 7), and change in metabolic states (8, 9). All or some these processes may be regulatory mechanisms involving calmodulin, a ubiquitous and extraordinarily versatile Ca^{2+}-binding protein. Means and collaborators using indirect immunofluorescence technique found that calmodulin may be associated with the chromosome-to-pole microtubules and may regulate the assembly/disassembly of microtubules in the mitotic apparatous of cells in culture (10, 11). Using a calmodulin antagonist, we found that calmodulin may be involved in the initiation of DNA synthesis in cultured cells (12).

In order to further define the relationship between the cell progression in cell division cycle and change in calmodulin levels of cultured cells,

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The abbreviations used are: W-7, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide
CHO, Chinese hamster ovary

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we determined the calmodulin content during the cell cycle and examined the effect of calmodulin antagonist, W-7 (13) on the cell progression of Chinese hamster ovary (CHO-K_1).

MATERIALS AND METHODS

Materials : N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) was synthesized according to the methods of Hidaka et al. (13) or was purchased from Rikaken Co., Ltd. (Nagoya, Japan).

Cells and cell culture : CHO-K_1 cells were cultured in Ham's F-12 medium supplemented with 5% fetal bovine serum at 37°C in a 5% CO_2 humidified atmosphere. Synchronized cells were prepared in the following two ways. Mitotic cells were prepared by the minor modification of mitotic shake procedure by Terashima and Tolmach (14). Cells were cultured in F-12 medium supplemented with 5% fetal bovine serum until reaching a semi-confluent monolayer, and then the medium was replaced with F-12 medium supplemented with 10% - 15% fetal bovine serum. At an appropriate period, the semi-confluent monolayer was vigorously shaken to separate the mitotic cells. Higher concentrations of fetal bovine serum, up to 20% in the medium, were effective for mitotic cells to release and to float from the monolayer (15). In order to prepare the cells in S phase, the mitotic cells were treated with 2.5 mM thymidine for 14 hr and then incubated in a fresh medium without drugs for another 3 hr. The plating efficiency for CHO-K_1 cells was more than 92%. Generation time for CHO-K_1 cells in this experimental condition was ascertained to be about 13 hr. These cultures were also ascertained to be in the G_1 phase of 6 hr, S + G_2 phases of 6 hr, and M phase of 1 hr, using the combination method of pulse labeling-mitosis procedure and excess thymidine block-mitosis procedure, described previously (12). Cell number and cell size were monitored at appropriate periods with a Coulter Counter and Channelyzer, respectively. The concentration of W-7 required to suppress the cell progression was dependent on the cell density and the cell density was maintained constant in all of the experiments.

Assay for the calmodulin : Cells were gently rinsed 4 times with cold phosphate buffered saline, detached with rubber policeman after addition of 2.0 ml of hypo-osmotic buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, and 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and then sonicated. The sonicate was centrifuged after addition of 500 μg of bovine serum albumin and trichloroacetate (final 5.0%), and the resulting precipitate was dissolved in 1.0 ml of 0.15 M Tris-HCl (pH 7.5). An aliquot was subjected to calmodulin assay. Calmodulin was assayed by its ability to activate calmodulin-dependent phosphodiesterase partially purified from hog brain cortex (16).

RESULTS AND DISCUSSION

The calmodulin content in CHO-K_1 cells at various phases of the cell cycle was determined using synchronized cells (Fig. 1). During the G_1 phase, calmodulin content was maintained at the lowest level of 250 ng/10^6 cells. When the mitotic cells entered S phase, the calmodulin content began to increase and reached a maximum level in late G_2 or M phase. Calcium ion or/and calmodulin are considered to play an important role in regulating the early DNA synthesis and the cell division phases of the cell cycle (5, 17).

Recently, we reported that calmodulin antagonist W-7 suppresses the cell

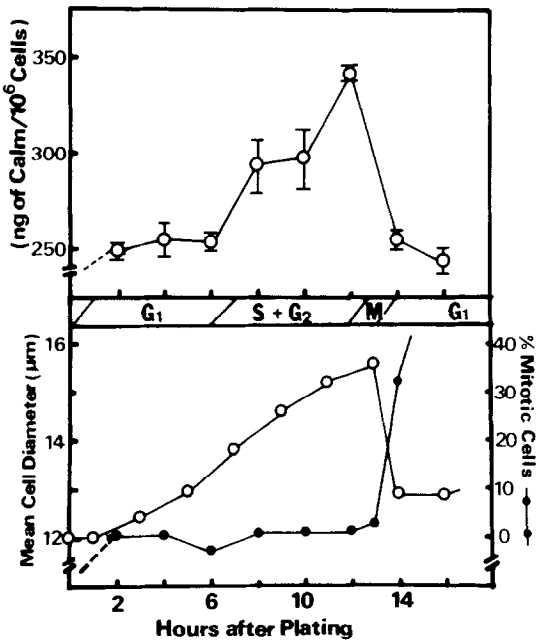

Fig. 1 Calmodulin content of CHO-K1 cells at various phases of the cell division cycle.
The method for the determination of calmodulin was described in the text. The cell size of CHO-K1 cells increased according to the cell progression in the cell cycle, and two peaks in cell size population were observed at 14 hr after the cell release. Cell division was observed at 1.5 hr and at 14 hr after cell release.

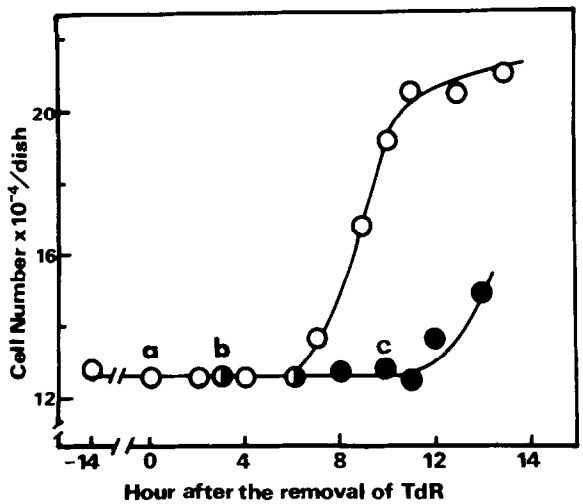

Fig. 2 Effect of W-7 on the cell cycle of CHO-K1 cells. 13.0 x 10^4 Mitotic cells were incubated in 60 mM plastic dishes containing F-12 medium with 2.5 mM thymidine without W-7 for 14 hr. The cells were washed and reincubated in a fresh conditioned medium containing no drug at 0 time (a,-o—o-). After 3 hr incubation, the cells were treated with 30 μM W-7 (b,-●—●-). "c" indicates the time for the removal of 30 μM W-7.

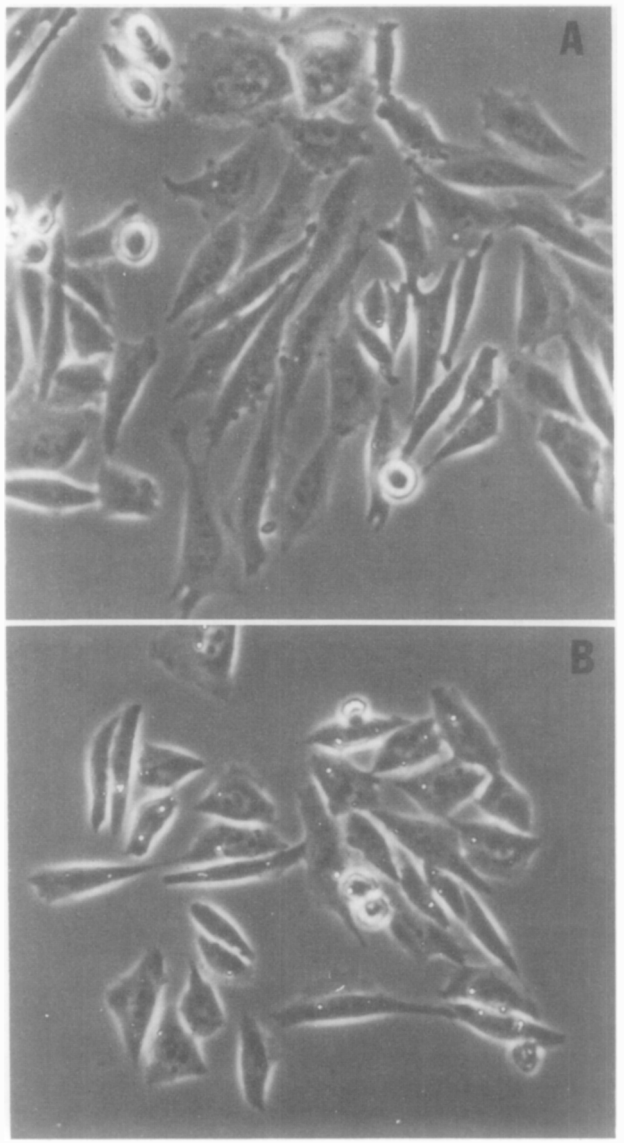

Fig. 3 Effect of W-7 on the morphology of CHO-K_1 cells.
All micrographs are 200x under phase-contrast microscope.
(A) No treatment; (B) treated with 30 μM W-7 for 26 hr; (C) treated with 100 ng/ml cholera toxin for 26 hr; (D) treated with 30 μM W-7 and 100 ng/ml cholera toxin for 26 hr.

proliferation of CHO-K_1 cells and that the cell cycle is blocked at the G_1-S boundary or early S phase, a time when the DNA synthesis is initiated (12). Therefore, the effect of calmodulin antagonist (W-7) on the G_2 or M phase was examined in the CHO-K_1 cell cycle. When the cells in S phase were treated with 30 μM W-7, cell division was not apparent until W-7 was removed from the medium and cell division began at 2 hr after this removal of W-7 (Fig. 2). Higher concentrations of W-7 were necessary to suppress the late G_2 or M phase,

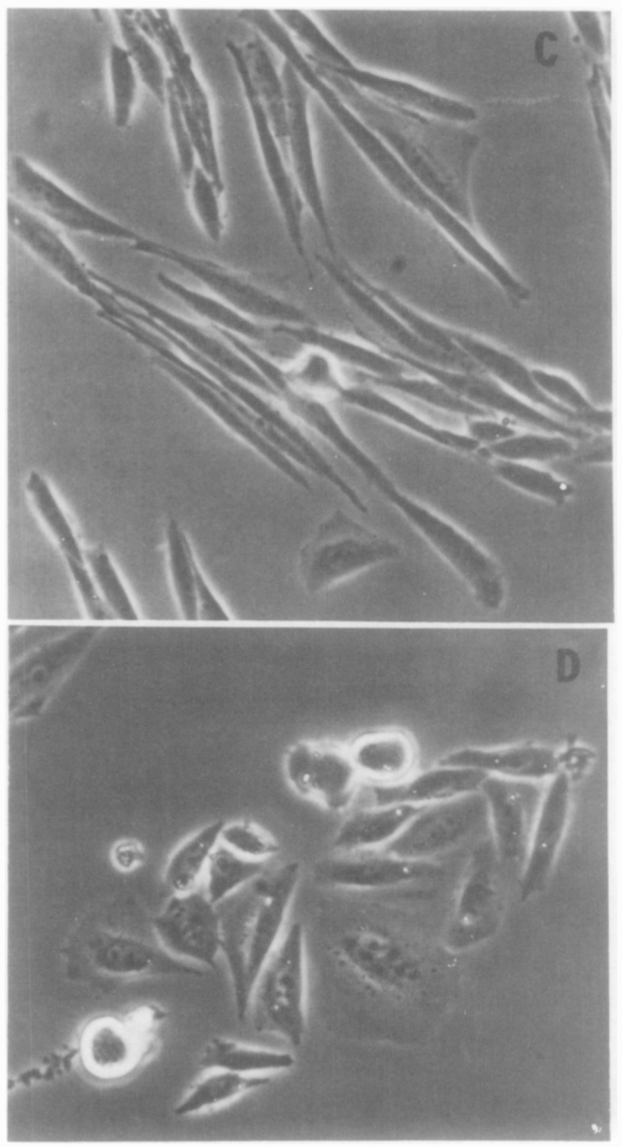

Fig. 3-- Continued.

compared with the case of the suppression of the early S phase. These results suggest that W-7 blocks the cell cycle at the late G_2 or the M phase, as well as at the G_1-S boundary phase. Moreover, calmodulin is not essential for CHO-K_1 cells to traverse the G_1 phase but is involved both in the initiation of DNA synthesis and in the mitosis.

Mitotic cells spread out in the first 3 hr following attachment to the plastic substratum. At 3 hr after plating, 100 ng/ml cholera toxin or/and 30 μM W-7 was added to the medium, and then the cells were incubated for another 26 hr. The control CHO-K_1 cells (no drug) assumed an epithelial form

with some knobs on the cell surface (Fig. 3). Following treatment with 30 μM W-7, the CHO-K_1 cells bacame more circular and compact with some knobs (Fig. 3). Similar results were obtained with colchicine at concentrations from 0.5 μM to 1.0 μM. As has been reported by Hsie and Puch (18), cholera toxin alters the shape of CHO-K_1 cells from epithelial to fibroblastic form, referred to as reverse-transformation. Thirty μM W-7 abolished the reverse-transformation caused by 100 ng/ml cholera toxin (Fig. 3). This effect induced by W-7 was also seen with 1.0 μM colchicine (data not shown). These results suggest that the inhibitory effect of W-7 on the initiation of DNA synthesis and mitosis of CHO-K_1 cells is mediated by disruption or rearrangement of microtubules.

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