CONCANAVALIN A INDUCES ENDOREDUPLICATION IN CULTURED MAMMALIAN CELLS

Shizuyo Sutou and Hiromi Shindo

Life Science Department, Nomura Research Institute
1600 Kajiwara, Kamakura, Kanagawa, Japan

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SUMMARY

Concanavalin A (Con A) induced endoreduplication in an established cell line, Don, of the Chinese hamster. The inducibility of Con A was inhibited by α-methyl-D-mannoside. When a secondary culture of kidney cells (CHK), which showed the contact-inhibition of growth, was used, there was an increase in spontaneous endoreduplication. CHK cells or some of them were more sensitive to Con A than Don cells, in which few spontaneous endoreduplications were observed. Mitotic shake-off after Con A treatment led to the higher ratio of endoreduplicated cells to normal mitoses, suggesting that endoreduplicating cells do not "round-up" and probably do not condense chromosomes through the cell cycle until M is reached.

INTRODUCTION

Endoreduplication is a mechanism which leads to exact karyotype doubling. Endoreduplication cell cycle consists of G_1, S_1, G_2, (M), G_3, S_2, G_4, and M (1). There is a wide variety of endoreduplication inducers, some of which are mutagens (2). When cells in late S or, mainly, in early G_2 are treated with inducers, some cells do not divide, but go into G_3, S_2, etc. (2, 3). Membrane fixation and/or disruption of the cytoskeleton was assumed to be a possible mechanism of endoreduplication induction (4). The finding that Concanavalin A (Con A) is one of the most potent inducers indicates that there is a close relationship between cell membrane and the induction of endoreduplication.

MATERIALS AND METHODS

Cells and Medium: An aneuploid Chinese hamster cell line, Don, was obtained through Dr. H. Kato at the National Institute of Genetics (Japan). Secondary cultures of fibroblast-like cells (CHK) from kidneys of male Chinese hamsters were available in our laboratory. Enriched Eagle's minimum essential medium (MEM) was used (4). Cells were grown as monolayers in plastic Petri dishes 60 mm in dismeter (Lux Scientific Corp., Thousand Oaks, Calif.), unless otherwise mentioned, in a CO_2 incubator.

Chromosome Preparations: Cells were treated with colcemid (Grand Island Biol. Co., Grand Island, N.Y.) at a concentration of 0.1 μg/ml for 2 hr prior to

chromosome preparation. The other procedures were as given in the previous report (4).

Chemicals: Con A was obtained from Sigma Chemical Co., St. Louis, Mo. and α-methyl-D-mannoside (α-MM) was from Fluka AG, Buchs SG, Swit.

RESULTS AND DISCUSSION

Con A is capable of inducing endoreduplication (Table I). The induction seemed to be proportional to levels up to 500 μg/ml, and reached a plateau at higher levels. Receptor sites to Con A on cell surfaces are likely to be saturated at 1,000 μg/ml.

Table I

Induction of Endoreduplication by Con A at Different Concentrations

Concentrations (μg/ml)	No. of endoreduplicated cells per 1,000 mitoses examined
0	0
62.5	8
125	10
250	20
500	42
1,000	44

10^5 Don cells per dish were plated. After 2 days, cells were treated with Con A for 4 hr, washed twice with serum free MEM, and cultured for 27 hr in fresh MEM. 0.1 μg/ml of colcemid was added 2 hr before the preparation of chromosome specimens. Cells having diplochromosomes were counted as endoreduplicated cells.

Table II shows the time course of the expression of endoreduplication. When a similar experiment was carried out using 4-nitroquinoline 1-oxide, the maximum ratio was observed after 27 hr (5). When Con A was used, the maximum was reached after 29 hr. This time difference is not significant. Since the generation time of Don cells is 12 hr, it takes more than 2 generation times to express endoreduplication. The fairly long period necessary for the expression is mainly due to S_2, ca. 18 hr (2).

When the ploidy of endoreduplicated cells was examined, both pseudodiploid (less than 30 chromosomes per cell) and pseudopolyploid (more than 31 per cell)

Table II

Expression of Endoreduplication after Con A Treatment.

Expression time (hr)	No. of endoreduplicated cells per 1,000 mitoses examined	No. of cells of which diplochromosomes are ≦30	>30
21	9	9	0
25	36	24	12
27	42	25	17
29	50	37	13
31	20	18	2

Don cells were treated with 500 μg/ml of Con A for 4 hr. Other conditions were as given in the legend to Table I. Expression time indicates the time between the cessation of Con A treatment and the beginning of the chromosome preparation.

Table III

Inhibition of Action of Con A by α-MM

Treatments	Concentrations (μg/ml)	No. of endoreduplicated cells per 1,000 mitoses examined
Control		0
Con A	250	35
α-MM	1,000	2
[Con A + α-MM]	250 + 1,000	8
α-MM	1,500	1
[Con A + α-MM]	250 + 1,500	5
α-MM	2,000	0
[Con A + α-MM]	250 + 2,000	2

Same as Table I, except that chromosomes were prepared 28 hr after the cessation of Con A treatment.

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cells were found to be endoreduplicated, but the appearance of endoreduplicated pseudopolyploid cells seemed to be slightly delayed.

α-MM effectively inhibited the action of Con A (Table III).

Don cells do not show contact-inhibition of growth. A strain of CHK cells mainly consisted of diploid cells and trisomy cells. These cells showed con-

tact-inhibition of growth. When CHK cells were treated with Con A, they revealed higher ratios of endoreduplicated cells to normal cells than those of Don cells (Table IV). Another feature of this strain is the fairly high spontaneous occurrence of endoreduplication.

Another CHK cell strain, derived from a kidney of a different Chinese hamster, showed few spontaneous endoreduplications, and seemed less sensitive

Table IV

Endoreduplication Induction by Con A in CHK Cells

Concentrations (μg/ml)	No. of mitoses examined	No. of cells of which chromosomes are			No. of endoreduplicated cells (%)	
		<30	30<,≦60	>60		
0	1,000	966	21	1	12	(1.2)
25	1,000	966	14	0	20	(2.0)
50	1,000	916	28	2	54	(5.4)
100	505	438	8	0	59	(11.7)
150	295	240	15	0	40	(13.6)
200	500	360	4	3	133	(26.6)

10^5 CHK cells per dish were plated. After 2 days, cells were treated with Con A for 4 hr. Cells were fixed 29 hr after Con A treatment.

to Con A than the cells shown in Table IV.

The endoreduplication cell cycle, from G_1 to M, requires more than 3 generation times, 36 hr. During the extraordinarily long cell cycle, an endoreduplicating cell remains undivided, while a normal cell divides 2 to 3 times, giving rise to 4 to 8 progeny cells. It seems certain that the difference in the length of the cell cycle allows the ratio of endoreduplicated cells to normal mitoses to be lower, as compared with the ratio just after induction. This dilution effect is very likely to have caused the comparatively small number of cells having diplochromosomes. Conversely, if normal mitotic cells are selectively removed from cultures, the dilution effect becomes small and the ratio of endoreduplicated cells would be high.

Mitotic cells "round-up" and are readily removed from the substrate by

Table V

Effect of Washing on the Ratio of Endoreduplicated Cells to Total Mitoses.

Treatments	Washing	No. of endoreduplicated cells per 1,000 mitoses examined.
Control	gently, twice	0
Con A	gently, twice	8
Con A	Vigorously, twice	17
Con A	Vigorously, twice, then twice at 30 min intervals	24
Con A	Vigorously, twice, then 4 times at 30 min intervals	71
Con A	Vigorously, twice, then 6 times at 30 min intervals	66

2.5×10^5 Don cells were plated in a dish 100 mm in diameter. After 2 days, medium was collected as conditioned medium for washing. Cells were treated with 100 µg/ml of Con A for 4hr. Gentle washing was carried out as follows: Medium was removed, 5 ml of the conditioned medium was poured gently into a dish, which was rotated a few times, and the medium was aspirated out. Vigorous washing was carried out as follows: medium was removed, 5ml of the conditioned medium was blown against monolayers of cells for 3 times, and the medium was removed. Fresh MEM was added after washing Con A. The medium was stored and replaced after further washings. The other conditions were as given in the legend to Table I.

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shaking (6). This behavior was the basis of determining whether endoreduplicating cells show the "round-up" between G_2 and G_3 or remain attached to the surface of the dish throughout the cell cycle until reaching M. The data in Table V indicate that vigorous washing resulted in a high increase of the ratio of endoreduplicated cells. These results seem to show that normal mitotic cells were more selectively removed by washing them from mixed cultures of normal and endoreduplicating cells. In other words, endoreduplicating cells did not "round-up" and become attached more firmly to the substrate during the washing period, suggesting that condensation of chromosomes, a prerequisite to mitosis, did not occur in mid-endoreduplication.

Preliminary experiments showed that N^6, $O^{2'}$ -dibutyryl adenosine 3', 5'-cyclic monophosphate was effective in suppressing the endoreduplication-inducing action of Con A. Binding of Con A to cell surfaces may impair the produc-

tion of cyclic nucleotides, cause the disturbance of cell cycle, and lead to endoreduplication.

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