Cell Prolif. 1999, **32**, 337–349

Lack of synchrony among multiple nuclei induces partial DNA fragmentation in V79 cells polyploidized by demecolcine

K. Fujikawa-Yamamoto*, C. Ohdoi*, H. Yamagishi*, Z. -p. Zong*, M. Murakami* and N. Yamaguchi†

**Division of Basic Science, Research Institute of Medical Science and †Department of Serology, Kanazawa Medical University, Uchinada, Ishikawa, Japan*

(*Received* 9 *April* 1999; *revision accepted* 2 *August* 1999)

Abstract. The nuclear morphology of polyploidized cells was examined in V79 Chinese hamster cells polyploidized by demecolcine or K-252a, inhibitors of spindle fibre formation and protein kinases, respectively. A variety of nuclear morphologies, including multinuclei, were observed in V79 cells polyploidized by demecolcine but not by K-252a, which produced mononuclear cells. A lack of synchrony in the nuclear cycle was observed among nuclei in multinuclear polyploidized cells. Partial DNA fragmentation, defined as DNA fragmentation of a nucleus in a multinuclear cell, was detected using the TUNEL method in V79 cells polyploidized by demecolcine but not by K-252a. Apoptosis occurred earlier in cell populations treated with demecolcine than in these treated with K-252a once the drugs were removed from the medium, suggesting that polyploidized cells with separate nuclei tend to apoptose earlier than those with mononuclei.

INTRODUCTION

Polyploidization by some cell-cycle inhibitors is carried out through DNA re-replication without cytokinesis (Kung, Sherwood & Schimke 1990, Lanks & Lehman 1990, Ishida *et al.* 1991, Lopes *et al.* 1993, Ishida *et al.* 1994, Okuda & Kimura 1988, Palitti & Rizzoni 1972, Yoshida *et al.* 1990, Usui *et al.* 1991, Traganos *et al.* 1994). The nuclear morphology of polyploidized cells may be the result of the mechanism by which the inhibitors act. A variety of nuclear morphologies in cells polyploidized by cell-cycle inhibitors have been thoroughly investigated; however, the mechanism of nuclear formation is not well understood (Dey, Paweletz & Ghosh 1989). It has been reported that many micronuclei were formed in cells treated with demecolcine through reconstruction of nuclear envelopes around scattered chromosomes (Chai, Weinfeld & Sandberg 1978, Dey *et al.* 1989)

Demecolcine (Colcemid), a colchicine structural analogue, reversibly binds to tubulin, depolymerizes microtubules and prevents spindle-fibre formation in the M phase (Inoue 1981, Salmon, McKeel & Hays 1984). Therefore, cells are tentatively arrested in the M phase under appropriate demecolcine conditions.

Correspondence: Dr K. Fujikawa-Yamamoto, Division of Basic Science, Research Institute of Medical Science, Kanazawa Medical University, Uchinada, Ishikawa 9200293, Japan. E-mail: fujikawa@kanazawa-med.ac.jp

K-252a, a protein kinase inhibitor with a molecular structure similar to that of staurosporine (Kase *et al.* 1987, Hashimoto 1988), polyploidizes most cultured cells, although the enzymes specifically inhibited by this drug and the mechanism triggering polyploidization are still unknown (Zong *et al.* 1994). Usui *et al.* (1991) suggested that K-252a polyploidized rat diploid fibroblasts (3Y1 cell line) arose through G_2–G_1 bypass.

A prior study reported that the V79 cell line was polyploidized by demecolcine, as well as K-252a (Fujikawa-Yamamoto, Teraoka & Odashima 1993). Although demecolcine effectively induced apoptosis in V79 cells at a critical concentration, the lowest effective concentration (Fujikawa-Yamamoto *et al.* 1993, 1994), this drug polyploidizes the cells at a concentrations at which it arrested cells in M phase.

Apoptosis is a mode of cell death that involves the active participation of the cell in its own destruction and is distinct from necrosis. The general characteristics of apoptosis consist of distinct morphological and biochemical changes that include cell shrinkage, loss of plasma-membrane microvilli, cell surface blebbing, segmentation of the nucleus and extensive degradation of DNA into oligonucleosomal-sized fragments (Kerr, Wyllie & Currie 1972, Wyllie, Kerr & Currie 1980).

Attention in this study was focused on the fact that the nuclei in a given polyploid cell containing more than two nuclei are not always synchronized. When one nucleus of a multinuclear polyploid cell is out of synchrony with another nucleus with respect to the nuclear cycle, the cell might undergo apoptosis, in whole or part.

MATERIALS AND METHODS

Cells

V79 cells (Chinese hamster lung cell line) were maintained as monolayer cultures in Leibovitz's L15–Ham's F10 mixture (7 : 3) supplemented with 10% fetal calf serum (M.A. Bioproducts, MD, USA), streptomycin (100 μg/ml) and penicillin (50 units/ml) at 37°C in a humidified atmosphere of 5% CO_2. The cells were cultured at low density.

Drug treatments

Exponentially growing V79 cells were inoculated into culture dishes (60 mm diameter, Nalge Nunc International, IL, USA) and Chamber-Slides (9.45 cm^2, Nalge Nunc International Co.) at densities of 1 × 105 cells/dish and 1 × 104 cells/chamber slide, and incubated for 36 h, during which the medium was changed once. The cells were exposed to demecolcine (Sigma, St Louis, MO, USA) or to K-252a (Funakoshi, Tokyo, Japan) at various concentrations, and incubated at 37°C. The cells were prepared at various times for determining DNA histograms and nuclear morphology.

In the recovery experiments, exponentially growing V79 cells were seeded in 60 mm diameter culture dishes and cultured for 12 h. The cells were exposed to demecolcine or K-252a at various concentrations and incubated for 72 or 96 h. Then the cells were washed twice with medium (drug-free) and incubated again in medium. At various times, the cells were harvested and used for DNA gel electrophoresis and DNA histograms determination.

Cell preparation for flow cytometry (FCM)

After the drug treatment, V79 cells were washed twice with $PBS^{(-)}$ (divalent cation-free phosphate-buffered saline) and trypsinized (0.17% trypsin, 30 mM EDTA). The cells were resuspended in medium, centrifuged and fixed with 20% ethanol at 4°C. Then the cells were

resuspended in 0.17% RNase (Type IIa, Sigma)/$PBS^{(-)}$ and incubated for 15 min at room temperature. The V79 cells were stained red with PI (propidium iodide, 50 μg/ml) just before measurement. Under these staining conditions, the signal resulting from residual double-stranded RNA is negligible and the relative intensity of red fluorescence corresponds to the DNA content (Krishan 1975).

Flow cytometry (FCM)

The red fluorescence of PI-stained V79 cells was measured using a FACSort (Becton Dickinson Immunocytometry Systems, USA). The fluorescence of individual cells irradiated with a focused laser light at 488 nm was separated optically through a dichromatic mirror and a cut-off (long-pass) filter, and it was detected using a photomultiplier. DNA histograms were determined from the relative intensity of red fluorescence.

Observation of nuclear morphology

V79 cells after the drug treatments on chamber slides were washed twice with $PBS^{(-)}$ and fixed with methanol. The cells were stained with Giemsa solution. (Nacalai Tesque, Kyoto, Japan) and viewed under a microscope (BX50–34-FLAD1, Olympus, Tokyo, Japan) equipped with a camera system. The photographic images were stored in a personal computer (Apple Macintosh) and printed at a given magnification.

Agarose gel electrophoresis of DNA fragments

Aliquots of V79 cells (1×10^6 cells) were fixed with 20% ethanol at 4°C. The cells were centrifuged, resuspended in 0.2 ml lysis buffer (0.5% sodium *N*-lauroylsarcosinate, 0.1 mg/ml RNase, 0.1 mg/ml proteinase K, 10 mM EDTA-Na_2 and 50 mM Tris–HCl, pH 7.8) and incubated for 4 h at 37°C. The cell lysate was mixed with 0.3 ml sodium iodide solution (6 M NaI, 0.5% sodium *N*-lauroylsarcosinate, 10 mg/ml glycogen, 13 mM EDTA-Na_2 and 26 mM Tris–HCl, pH 8.0) and incubated for 15 min at 60°C. DNA was precipitated with isopropanol, dried with diethylether and dissolved in 10 μl loading buffer (20% glycerol, 1 mM EDTA-Na_2, 10 mM Tris–HCl, pH 8.0). The DNA solutions were loaded into the wells of 1.5% agarose gels and electrophoresis carried out in TAE (Tris–acetate–EDTA) electrophoresis buffer. DNA was visualized by ethidium bromide staining.

Terminal deoxynucleotidyl transferase nick end-labelling (TUNEL) assay

The V79 cells treated by the drugs on chamber slides were prefixed with 4% formalin/neutral buffer and fixed with 70% ethanol at 4°C. Apoptosis (indicated by fragmented DNA) was detected using an Apop Tag Direct *In Situ* Apoptosis Detection Kit – Fluorescein (Oncor, MD, USA) using the TUNEL (terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end-labelling) method. Briefly, DNA nicks were labelled green by adding fluorescein labelled-dUTP to 3OH ends of DNA fragments through terminal deoxynucleotidyl transferase. Green fluorescence from cells with fragmented DNA was observed using a fluorescence microscope (BX50–34-FLAD1, Olympus Co.) equipped with a differential interference device and a digital camera system (Eastman Kodak, Rochester, NY, USA). Cells were counter stained with PI (5 μg/ml).

RESULTS

To examine how demecolcine and K-252a affect the cell cycle of V79 cells, DNA histograms were obtained by flow cytometry for the cell populations treated with the drugs at various

concentrations (Figure 1). At a lower concentration (81 nM) of demecolcine, progressive increase of the polyploid cell population was observed with a sub-G_1 population suggesting apoptosis (Figure 1d–f). At a higher concentration (270 nM) of demecolcine, V79 cells polyploidized progressively without clear sub-G_1 peaks (Figure 1g–i). Demecolcine at 27 nM barely affected the histogram patterns (data not shown) and cell growth was not inhibited by demecolcine at this concentration, but was inhibited at 81 and 270 nM (data not shown).

V79 cells were also polyploidized without an increase of sub-G_1 population by K-252a, as well as 270 nM demecolcine (Figure 1a,b). It should be noted that polyploid V79 cells with 16C DNA content appeared earlier (48 h) in cells treated with K-252a than in cells treated with demecolcine (Figure 1b,h). It was concluded that demecolcine induced apoptosis and polyploidization in V79 cells, whereas K-252a induced polyploidization alone.

To ascertain the nuclear morphology of polyploidized V79 cells, the cells were exposed to demecolcine or K-252a for 48 h, stained with Giemsa solution and examined by microscopy (Figure 2). Note that both less damage and a higher polyploidy of the cells was seen after the treatment for 48 h. Various nuclear morphologies, such as a torus-like nucleus (Figure 2b), mininuclei (c,d), multinuclei (e) and mononucleus (f), were observed in V79 cells polyploidized by demecolcine, whereas multilobed nuclei (g,h) and mononuclei (i) were observed in cells polyploidized by K-252a. Thus, various nuclear morphologies were induced in V79 cells by demecolcine, and the nuclear morphology was distinct from that induced in K-252a-treated V79 cells. To explain how the various nuclear morphologies are formed in V79 cells polyploidized by demecolcine, chromosome localization was examined (Figure 3). The polyploid chromosomes were located in a circle (Figure 3b,d), several groups (c), a dispersed form (d) or massed at the centre (e,f), implying that they would form a torus-like nucleus, multinuclei, mininuclei or mononuclei, respectively, if the nuclear membranes were formed by wrapping around each chromosome population (Chai *et al.* 1978, Dey *et al.* 1989).

To check whether or not intracellular synchronization in the nuclear cycle is maintained among nuclei of multinuclear polyploid cells, some of the nuclei-forming chromosomes were examined in demecolcine-treated V79 cells (Figure 4). Several nuclei of multinuclear cells (indicated by arrows in Figure 4) differed from each other in the degree of chromosome condensation, implying asynchrony of the nuclear cycle of these nuclei. From these results, it was postulated that when at least one nucleus of multinuclear cells markedly differs from the others in the nuclear cycle, DNA fragmentation might occur in some or all of the multinuclei.

In order to test the hypothesis that partial DNA fragmentation might occur in multinuclear polyploid cells, the TUNEL assay was used to detect DNA fragmentation as a marker of apoptosis in V79 cells polyploidized by demecolcine or K-252a. Figure 5 shows representative microphotographs of V79 cells exposed to demecolcine (Figure 5b,c,f,g,j,k) and K-252a (Figure 5d,h,l) for 72 h. Note that cell debris appeared in the histograms at 72 h after the drug addition. DNA fragmentation was observed in some but not all nuclei in multinuclear polyploid V79 cells treated with demecolcine (Figure 5j,k). K-252a, which led only to mononuclear polyploidy and did not induce any partial apoptosis (Figure 5l). These results suggest that some of the nuclei in multinuclear polyploid cells may undergo DNA fragmentation, which would then lead to apoptosis of the whole cell. To examine whether apoptosis was induced in V79 cells polyploidized by demecolcine or K-252a after the drugs were removed, DNA fragmentation in the cell population before and after the drug removal was examined by agarose gel electrophoresis (Figure 6). In the polyploidizing process, only a small amount of DNA fragmentation was detected. A striking difference between demecolcine and K-252a was observed after the drug removal. When V79 cells were released

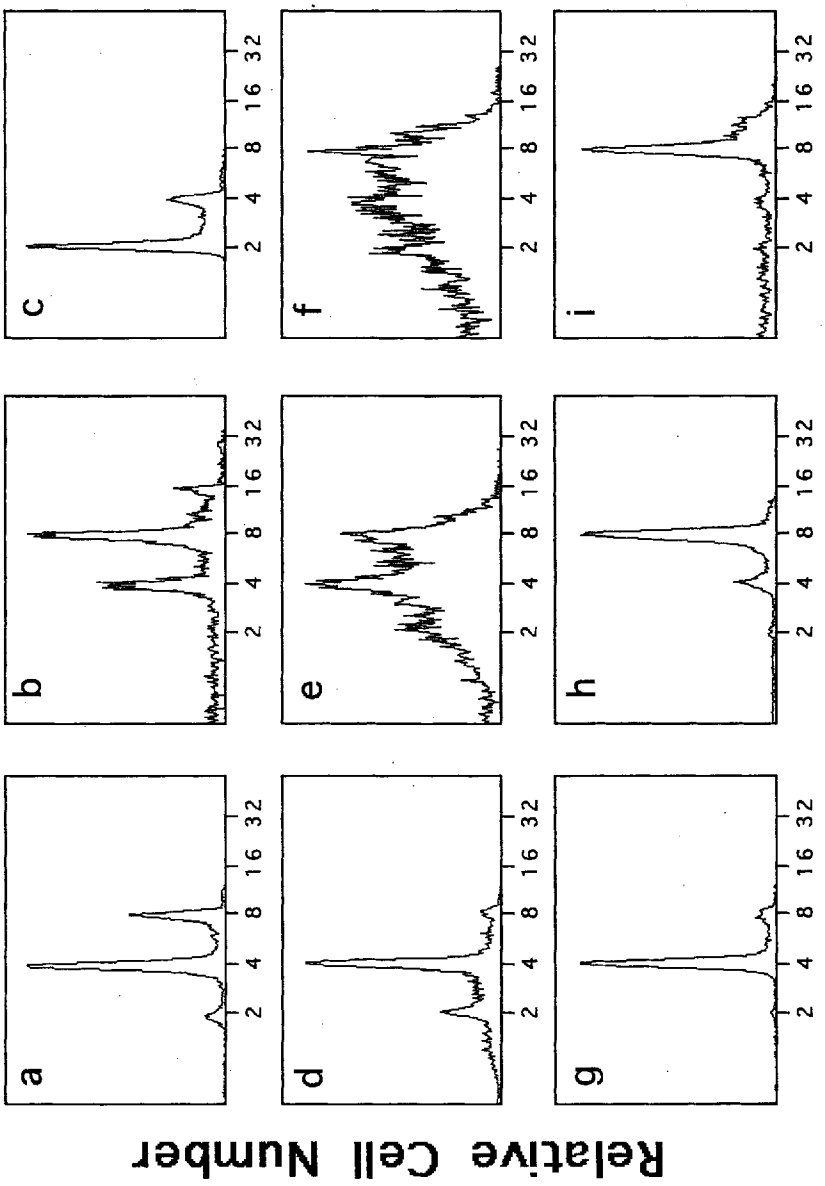

Figure 1. DNA histograms of V79 cells after addition of demecolcine or K-252a. Exponentially growing V79 cells were exposed to demecolcine at 0 (c), 81 (d–f) or 270 nM (g–i) or K-252a at 500 nM (a,b), and then harvested after 24 (a,d,g), 48 (b,e,h) or 72 h (f,i). The cell suspensions were stained red with PI and the histograms of the relative intensity of red fluorescence (DNA histograms) were obtained. The ordinate represents the relative cell number, the abscissa the relative DNA content (log scale). Histograms were normalized to have roughly equal maximum peak heights.

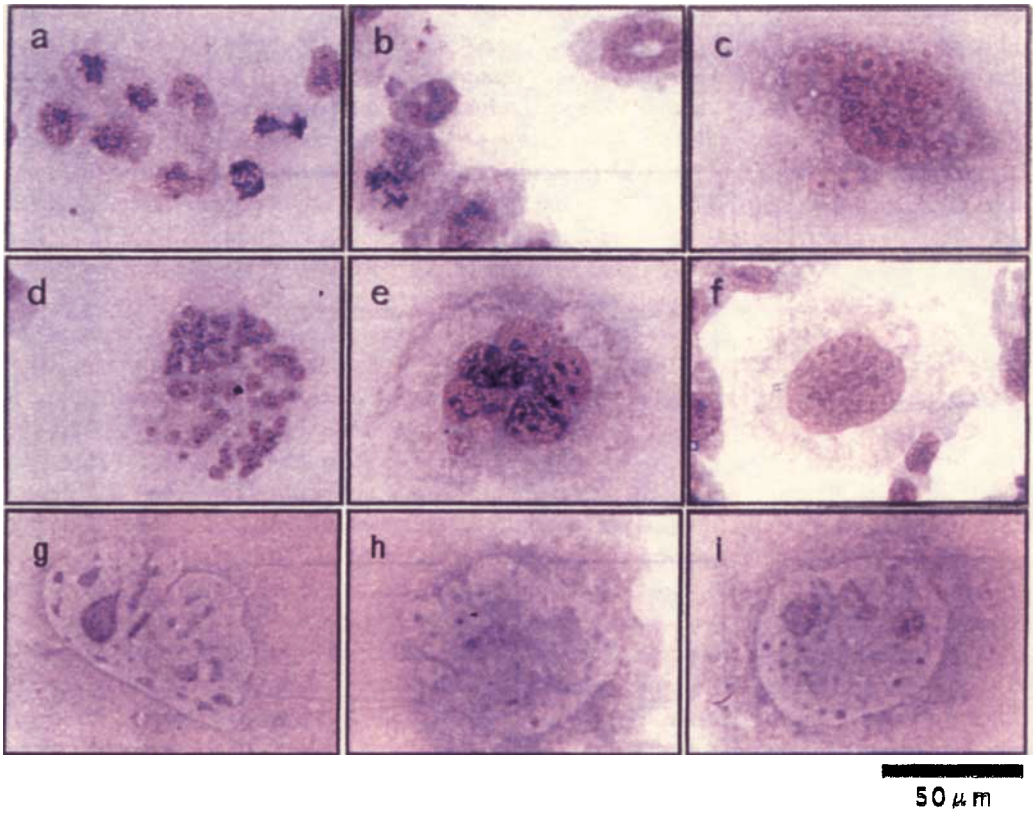

Figure 2. Various nuclear morphologies: photomicrographs of V79 cells in the presence of demecolcine or K-252a. Exponentially growing V79 cells were exposed to demecolcine at 0 (a) or 81 nM (b–f) or K-252a at 500 nM (g,h,i) for 48 h. The cells were stained with Giemsa solution and photographs of nuclear morphology were taken using a camera attached to a microscope. Various nuclear morphologies, such as a torus-like nucleus (b), mininuclei (c and d), multinuclei (e) and mononucleus (e), were observed in V97 cells polyploidized by demecolcine, whereas multilobed muclei (g and b) and mononuclei (i) were shown by K-252a.

from the 72-h exposure to demecolcine or K-252a, apoptosis occurred earlier in the demecolcine-treated cells than in the K-252a treated cells.

Figure 7 shows a changes of DNA histograms of V79 cells after the removal of demecolcine or K-252a. An appreciable amount of sub G1 cells were observed at 24 and 48 h in demecolcine-treated V79 cells. It thus seems that apoptosis was induced earlier in multinuclear polyploidy than in mononuclear polyploidy, when the cell-cycle inhibitors were removed from the system.

DISCUSSION

Demecolcine reversibly binds to tubulin, depolymerizes microtubules and inhibits the microtubule formation (Inoue 1981, Salmon *et al.* 1984). This drug strongly affects the M phase and tentatively arrests cells at this point in the cell-cycle. As microtubules determine the localization of organelles through microtubule-based motor proteins that transport

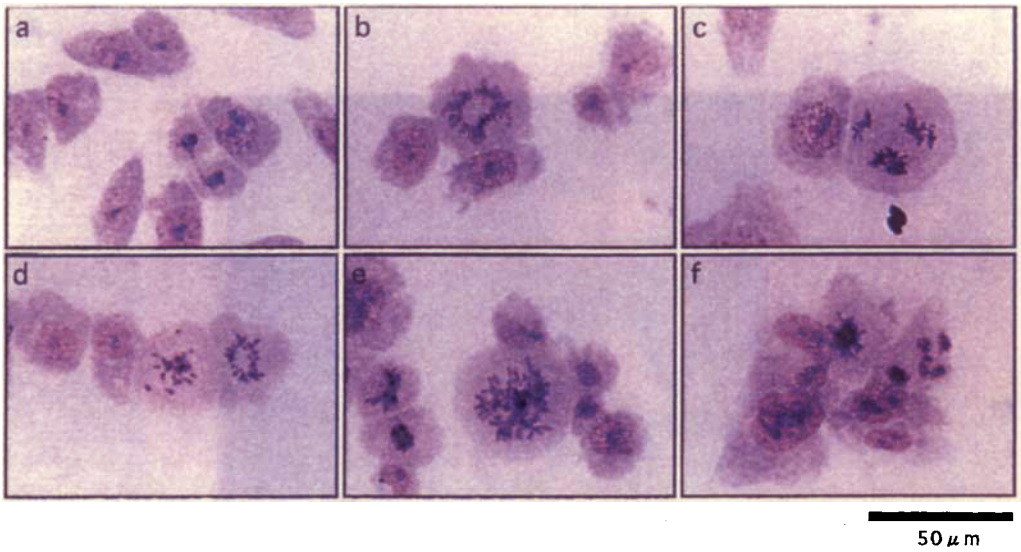

Figure 3. Chromosome localization: photomicrographs of V79 cells in the presence of demecolcine. Exponentially growing V79 cells were exposed to demecolcine at 0 (a) or 81 nM (b–f) for 48 h. Cells were stained with Giemsa solution and photographs showing the chromosome localization were taken using a camera attached to a microscope. Various chromosome localizations were observed, such as circle (b and d), several groups (c), a dispersed form (d) or massed at the centre (e and f), implying that they would form a torus-like nucleus, multinuclei or mononuclei, respectively.

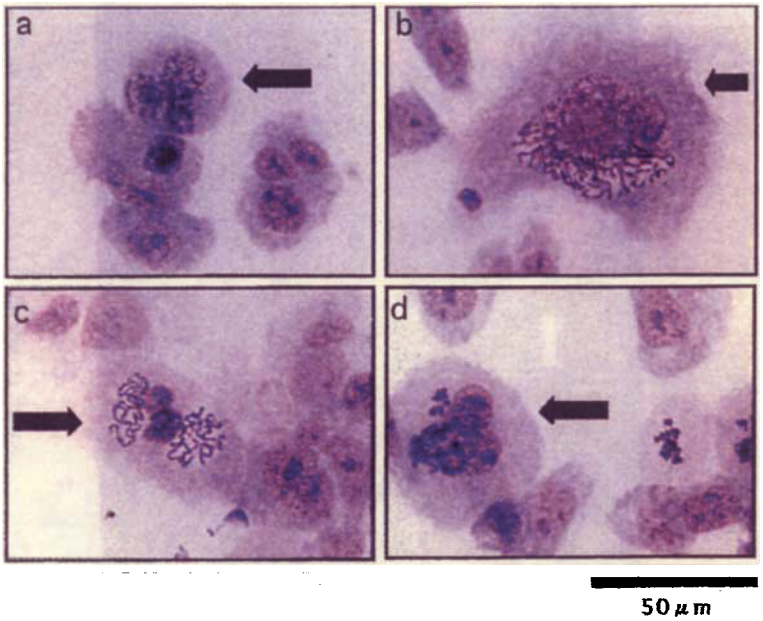

Figure 4. Examples of asynchrony in the nuclear cycle among nuclei in multinuclear cells: photomicrographs of V79 cells after demecolcine treatment. Exponentially growing V79 cells were exposed to demecolcine at 81 nM and then harvested after 48 h. Cells were stained with Giemsa solution and photographs were taken using a camera attached to a microscope. Several nuclei of multinuclear cells indicated by arrows differed from each other in the degree of chromosome condensation, implying asynchrony of the nuclear cycle of these nuclei.

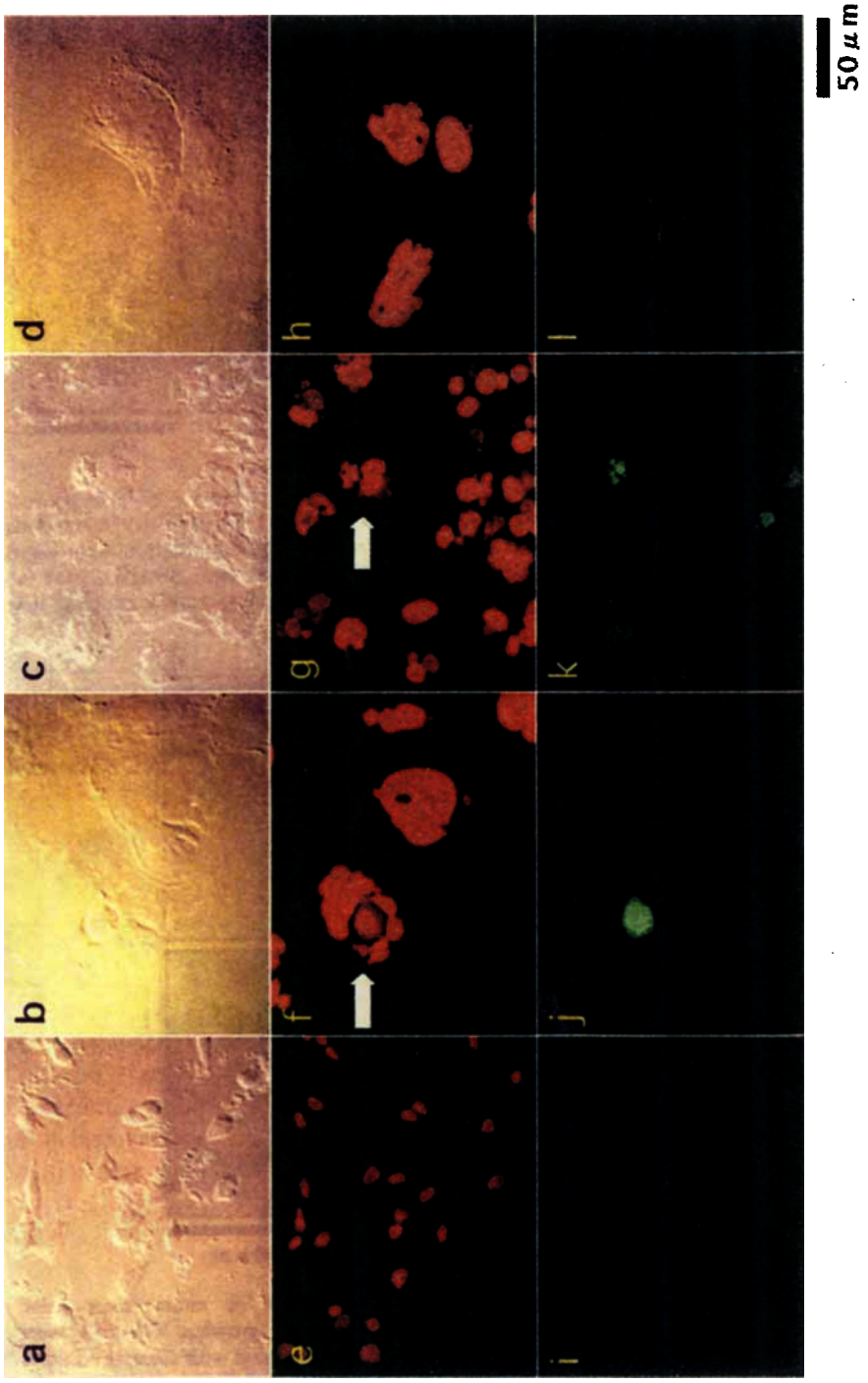

Figure 5. Evidence of partial apoptosis: photomicrographs of V79 cells after demecolcine or K-252a treatment. Exponentially growing V79 cells on chamber slides were exposed to demecolcine at 0 (a,e,i), or 81 nM (b,c,f,g,j,k) or K-252a at 500 nM (d,h,l) for 72 h, and then fixed with 4% formalin and 70% ethanol. After 24 h of incubation (a,e,i), unexposed cells were similarly fixed. 3OH ends of DNA were labelled with fluorescein-labelled dUTP, and detected using a fluorescence microscope (i,j,k,l). PI was used as the counter-stain (e,f,g,h). The same fields viewed with the fluorescence microscope (i,j,k,l) were viewed with a differential interference microscope (a,b,c,d, respectively). DNA fragmentation was observed in some but not all nuclei in multinuclear cells treated with demecolcine (j and k), but with K-252-a (l).

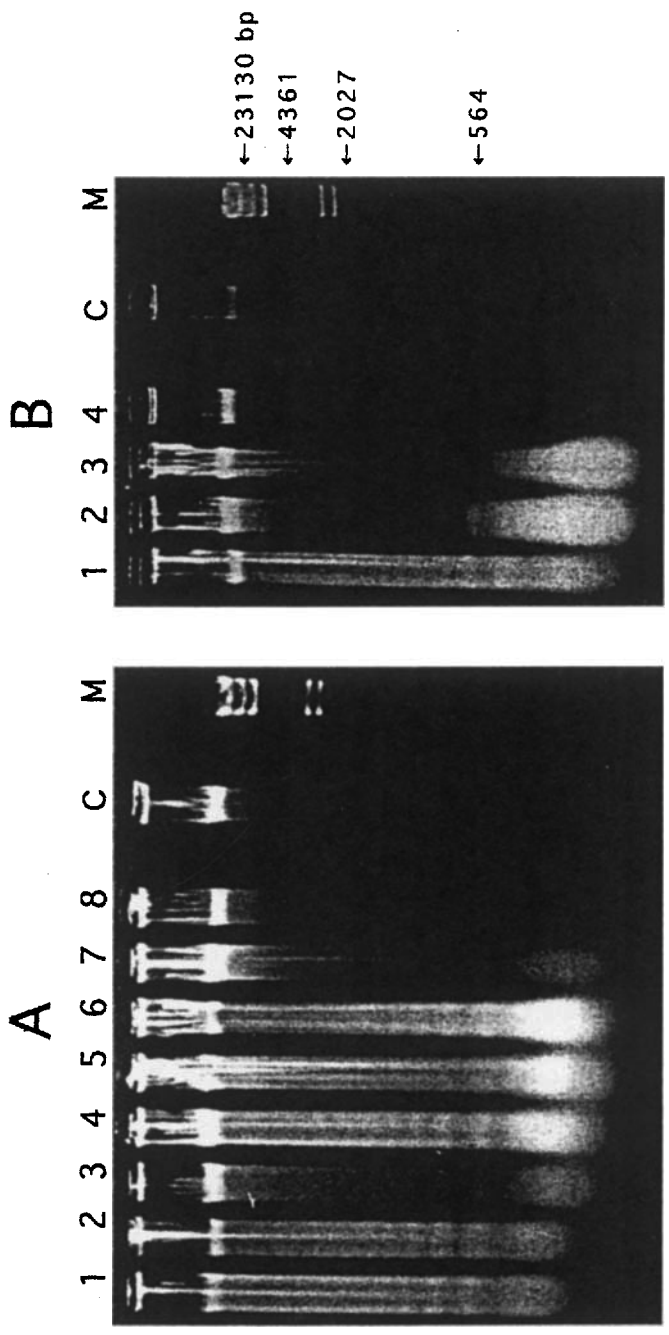

Figure 6. Electrophoretic profiles of DNA fragments from V79 cells after treatment with demecolcine (a) or K-252a (b). Exponentially growing V79 cells were exposed to demecolcine at 81 (2, 4, 6 and 8 in (a)) or 270 nM (1, 3, 5 and 7 in (a)), or K-252a at 500 (2, 4, 6 and 8 in (b)) or 1000 nM (1, 3, 5 and 7 in (b)), and then the medium was replaced by drug-free medium after 72 h. After further incubation for 0 (lanes 7 and 8), 24 (lanes 5 and 6), 48 (lanes 3 and 4) or 96 h (lanes 1 and 2), cells were harvested, DNA fragments were extracted by the sodium iodide method and agarose gel electrophoresis was carried out. Lane C containes the control. Lane M shows DNA markers of the indicated molecular weights (bp).

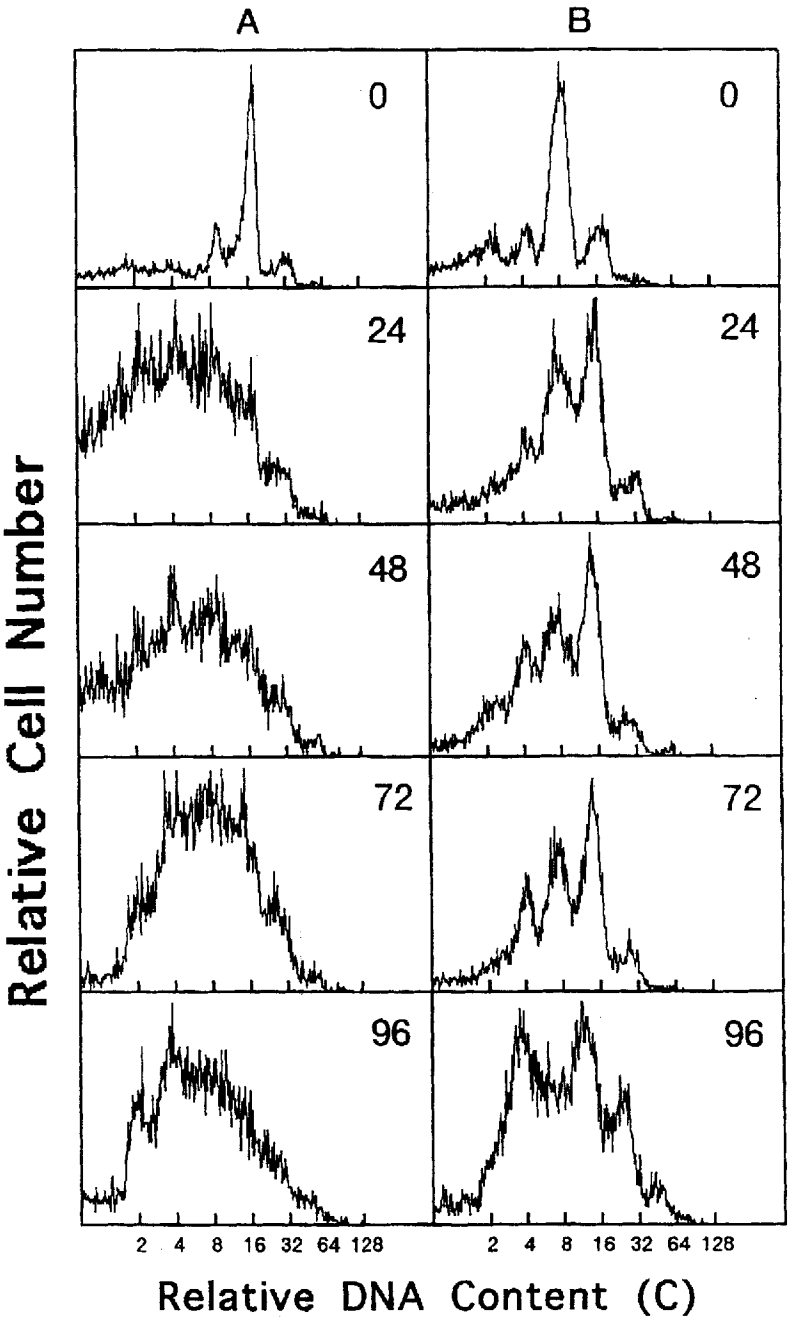

Figure 7. Changes in DNA histograms of V79 cell population treated with 270 nM demecolcine (a) and 800 nM K-252a (b) for 96 h. The drugs were removed at t = 0. Numerals in each histogram represents the time after the drug release. The abscissa represents relative DNA content (log scale). Histograms were normalized to have roughly equal maximum peak heights.

intracellularly organelles along microtubules (Vale, Reese & Sheetz 1985, Vallee *et al.* 1988, Bloom 1992, Skoufias & Scholey 1993), demecolcine will disorder mitosis, cytokinesis and intracellular organization during the cell-cycle. With longer treatment times at higher concentrations of demecolcine, apoptosis and polyploidization were induced in cells. Nuclear lamins are depolymerized by phosphorylation, and the nuclear envelope is then disassembled (Peter *et al.* 1991, Weise & Wilson 1993). Conversely, dephosphorylation leads to repolymerization of the nuclear lamina on the chromosome surface, and the nuclear envelope is reconstructed (Glass & Gerace 1990, Chaudhary & Courvalin 1993). Therefore, the nuclear membrane might wrap around each cluster of chromosomes. It is presumed that a reduced number of spindle fibres leads to a random distribution of chromosomes, and that when the nuclear envelope is reconstructed around chromosome groups without cytokinesis and multinuclei, mininuclei, torus-like nuclei or a mononucleus formed. Many studies of cybrids have revealed that binuclear cells made by cell fusion between two cells in different phases of the cell-cycle synchronize their nuclei, destroy one nucleus, or die (apoptosis) (Kato & Sandberg 1968, Rao & Johnson 1970, 1972). From these studies, it may be deduced that multinuclear cells die by apoptosis when they lose the synchrony of the nuclear cycle in their multiple nuclei. It was of interest to know whether or not apoptosis occurred sequentially in one nucleus after another in multinuclear polyploid cells or whether all nuclei simultaneously apoptosed. In the present study, it was demonstrated that DNA fragmentation occurred first in only one (or two) nuclei of a multinuclear polyploid cell. It is well known that apoptotic features include cell shrinkage, chromatin condensation, cell surface blebbing, extensive nuclear fragmentation, and apoptotic body formation (Kerr *et al.* 1972, Wyllie *et al.* 1980). Although some of these apoptotic features were routinely observed in polyploidized cells (Fujikawa-Yamamoto *et al.* 1997), the partial DNA fragmentation was observed only with the TUNEL assay. If partial DNA fragmentation indicates an early stage of apoptosis, these cells should be called partially apoptotic.

Careful observation of the differential interference microphotographs in Figure 5 seems to reveal that the cell surface morphology was changed in the region above the fragmented nucleus. At this stage, no possible mechanisms can be suggested by which partial DNA fragmentation causes a localized cell surface change. Spatial studies will be necessary to explain why partial apoptosis occurs in a cell.

The induction of apoptosis before and after drug removal seemed to occur by different mechanisms. The former may depend on cellular irregularity in the polyploidization process, while the latter may depend on the instability of polyploid cells in progression through the cell-cycle. V79 cells polyploidized by demecolcine seemed more unstable than cells polyploidized by K-252a. The small amount of DNA fragmentation observed in polyploidizing V79 cells may suggest that most of the cells did not die during their polyploidization.

More DNA fragmentation in polyploid cells was observed after the removal of the drugs from the medium, and the appearance of apoptosis after the drug removal occurred earlier in demecolcine-treated V79 cells than in K-252a-treated cells. After the removal of demecolcine, free tubulin polymerizes to form microtubules, mitotic spindles are re-formed and the cells try to progress through the cell-cycle. The disordered nuclei might have increased asynchrony, resulting in their apoptosis. After the removal of K-252a, polyploid V79 cells will recover their protein kinase activity, attempt to progress through the cell-cycle and transform their polyploid form (Fujikawa-Yamamoto *et al.* 1993). Although the mechanism of induction of apoptosis in polyploid cells after the removal of these drugs remains unknown in detail, the abnormal nuclei may fail functionally and thereby cause apoptosis.

It should also be borne in mind that the results of this study on the differing effects of demecolcine and K-252a may be a result of intracellular variation among V79 cells cultured under specific conditions.

ACKNOWLEDGEMENTS

This study was supported in part by a grant for the Project Research from High-Technology Center of Kanazawa Medical University (H98-1) and by a grant for the project of High-Tech-Research Center from the Ministry of Education, Science and Culture of Japan.

REFERENCES

BLOOM GS. (1992) Motor proteins for cytoplasmic microtubules. *Curr. Opin. Cell Biol.* **4,** 66.

CHAUDHARY N, COURVALIN JC. (1993) Stepwise reassembly of the nuclear envelope at the end of mitosis. *J. Cell Biol.* **122,** 295.

CHAI LS, WEINFELD H, SANDBERG AA. (1978) Effects of divalent-cation chalators and chloramphenicol on the spatial relationship of the nuclear envelope to chromatin in micronuclei of Chinese hamster cells. *J. Supermol. Struct.* **9,** 459.

DEY R, PAWELETZ N, GHOSH S. (1989) The behavior of centrosomes in multinucleate cells formed after colcemid treatment. *Eur. J. Cell Biol.* **48,** 227.

FUJIKAWA-YAMAMOTO K, TERAOKA K, ODASHIMA S. (1993) Hyperploidization of V79 cells by K-252a in comparison with demecolcine. *Cell Struct. Funct.* **18,** 449.

FUJIKAWA-YAMAMOTO K, TERAOKA K, ZONG Z, YAMAGISHI H, ODASHIMA S. (1994) Apoptosis by demecolcine in V79 cells. *Cell Struct. Funct.* **19,** 391.

FUJIKAWA-YAMAMOTO K, ZONG Z, MURAKAMI M, ODASHIMA S, IKEDA T, YOSHITAKE Y. (1997) Spontaneous polyploidization results in apoptosis in a Meth-A tumor cell line. *Cell Struct. Funct.* **22,** 399.

GLASS JR, GERACE L. (1990) Lamins A and C bind and assemble at the surface of mitotic chromosomes. *J. Cell Biol.* **111,** 1047.

HASHIMOTO S. (1988) K-252a, a potent protein kinase inhibitor, blocks nerve growth factor-induced neurite outgrowth and changes in the phosphorylation of proteins in PC12h cells. *J. Cell Biol.* **107,** 1531.

INOUE S. (1981) Cell division and the mitotic spindle. *J. Cell Biol.* **91,** 131.

ISHIDA R, MIKI T, NARITA T *et al.* (1991) Inhibition of intracellular topoisomerase II by antitumor bis (2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. *Cancer Res.* **51,** 4909.

ISHIDA R, SATO M, NARITA T *et al.* (1994) Inhibition of DNA topoisomerase II by ICRF-193 induces polyploidization by uncoupling chromosome dynamics from other cell cycle events. *J. Cell Biol.* **126,** 1341.

KASE H, IWAHASHI K, NAKANISHI S *et al.* (1987) K-252 compounds, novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinases. *Biochem. Biophys. Res. Commun.* **142,** 436.

KATO H, SANDBERG AA. (1968) Chromosome pulverization in Chinese hamster cells induced by Sendai virus. *J. Natl. Cancer Inst.* **41,** 1117.

KERR JF, WYLLIE AH, CURRIE AR. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26,** 239.

KRISHAN A. (1975) Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. *J. Cell Biol.* **66,** 188.

KUNG AL, SHERWOOD SW, SCHIMKE RT (1990) Cell line-specific differences in the control of cell cycle progression in the absence of mitosis. *Proc. Natl. Acad. Sci. USA* **87,** 9553.

LANKS KW, LEHMAN JM. (1990) DNA synthesis by L929 cells following doxorubicin exposure. *Cancer Res.* **50,** 4776.

LOPES NM, ADAMS EG, PITTS TW, BHUYAN BK. (1993) Cell kill kinetics and cell cycle effects of taxol on human and hamster ovarian cell lines. *Cancer Chemother. Pharmacol.* **32,** 235.

OKUDA A, KIMURA G. (1988) Commitment to ploidy conversion of 3Y1 cells during metaphase arrest by colcemid. *Cell Tissue Kinet.* **21,** 21.

PALITTI F, RIZZONI M. (1972) Experimental evolution of cell populations of Chinese hamster treated with colchicine. Induction of high degree of ploidy and a phase-specific lethal effect. *Int. J. Cancer* **9,** 510.

PETER M, HEITLINGER E, HANER M, AEBI U, NIGG EA. (1991) Disassembly of in vitro formed lamin head-to-tail polymers by CDC2 kinase. *EMBO J.* **10,** 1535.

RAO PN, JOHNSON RT. (1970) Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. *Nature* **225,** 159.

RAO PN, JOHNSON RT. (1972) Premature chromosome condensation: a mechanism for the elimination of chromosomes in virus-fused cells. *J. Cell Sci.* **10,** 495.

SALMON ED, MCKEEL M, HAYS T. (1984) Rapid rate of tubulin dissociation from microtubules in the mitotic spindle in vivo measured by blocking polymerization with colchicine. *J. Cell Biol.* **99,** 1066.

SKOUFIAS DA, SCHOLEY JM. (1993) Cytoplasmic microtubule-based motor proteins. *Curr. Opin. Cell Biol.* **5,** 95.

TRAGANOS F, GONG J, ARDELT B, DARZYNKIEWICZ Z. (1994) Effects of staurosporine on MOLT-4 cell progression through G2 and cytokinesis. *J. Cell. Physiol.* **158,** 535.

USUI T, YOSHIDA M, ABE K, OSADA H, ISONO K, BEPPU T. (1991) Uncoupled cell cycle without mitosis induced by a protein kinase inhibitor, K-252a. *J. Cell Biol.* **115,** 1275.

VALE RD, REESE TS, SHEETZ MP. (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* **42,** 39.

VALLEE RB, WALL JS, PASCHAL BM, SHPETNER HS. (1988) Microtubule-associated protein 1C from brain is a two-headed cytosolic dynein. *Nature* **332,** 561.

WEISE C, WILSON KL. (1993) Nuclear membrane dynamics. *Curr. Opin. Cell Biol.* **5,** 387.

WYLLIE AH, KERR JF, CURRIE AR. (1980) Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68,** 251.

YOSHIDA M, NISHIKAWA M, ABE K, HORINOUCHI S, BEPPU T. (1990) Effects of leptomycin B on the cell cycle of fibroblasts and fission yeast cells. *Exp. Cell Res.* **187,** 150.

ZONG Z, FUJIKAWA-YAMAMOTO K, TERAOKA K, YAMAGISHI H, TANINO M, ODASHIMA S. (1994) Potentiation of K-252a, a protein kinase inhibitor-induced polyploidization by cAMP in cultured fibrosarcoma cell line. *Biochem. Biophys. Res. Commun.* **205,** 746.