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Cell line-specific differences in the control of cell cycle progression in the absence of mitosis

(p34^{cdc2}/cyclin B/Colcemid/taxol/nocodazole)

ANDREW L. KUNG, STEVEN W. SHERWOOD, AND ROBERT T. SCHIMKE*

Department of Biological Sciences, Stanford University, Stanford, CA 94305

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ABSTRACT **This paper reports that there are major differences between mammalian cell lines in the propensity to progress into subsequent cell cycles when mitosis is inhibited with agents that disrupt the assembly of the mitotic spindle apparatus (Colcemid, nocodazole, and taxol). Human HeLa S3 cells, which represent one extreme, remain arrested in mitosis, with elevated levels of cyclin B and p34^{cdc2} kinase activity. In Chinese hamster ovary cells, at the other extreme, the periodic rise and fall of cyclin B levels and p34^{cdc2} kinase activity is only transiently inhibited in the absence of mitosis. The cells progress into subsequent cell cycles, without dividing, resulting in serial doublings of cellular DNA content. In general, the propensity to progress into subsequent cell cycles in the absence of mitosis appears to be species related, such that human cell lines remain permanently blocked in a mitotic state, whereas rodent cell lines are only transiently inhibited when spindle assembly is disrupted. We interpret these results to indicate that in mammalian cell lines there exists a checkpoint which serves to couple cell cycle progression to the completion of certain karyokinetic events. Furthermore, either such a checkpoint exists in some cell lines but not in others or the stringency of the control mechanism varies among different cell lines.**

Current understanding of the cell cycle is based upon models in which phase transitions are regulated by complex networks of biochemical events, many of which are highly conserved in all eukaryotes. The activation of protein complexes containing the p34^{cdc2} kinase is central to the transitions from G_1 to S and from interphase to mitosis (1–3). The proper temporal order of cell cycle processes is maintained by control mechanisms which ensure that the initiation of later cell cycle events is dependent on the completion of earlier events (4). The activation of p34^{cdc2} to initiate the events of mitosis, for example, is modulated by several gene products that serve to couple mitosis to the completion of DNA synthesis and repair, as well as nutrient state and cell size (4, 5). It has been hypothesized that control mechanisms also exist that serve to couple the exit from mitosis with the correct assembly of the spindle apparatus (2).

In this paper, we report that major differences exist between mammalian cell lines in the ability to proceed into the next cell cycle when mitosis is inhibited with agents that disrupt microtubule polymerization. These differences are correlated with differences in the modulation of p34^{cdc2} activity, as well as cellular concentrations of cyclin B, and indicate that mammalian cells differ significantly in the regulation of the transition from mitosis into subsequent cell cycles.

MATERIALS AND METHODS

Cell Culture and Drug Treatment. HeLa S3 and Chinese hamster ovary cells strain AA8 (CHO-AA8) were maintained in minimal essential media α, supplemented with 10% fetal bovine serum, gentamicin at 50 μg/ml, and 2 mM glutamine, buffered with 10 mM Hepes at pH 7.4, and incubated at 37°C in a 5% CO_2 atmosphere. Culture conditions for other cell lines used are summarized in Table 1. Cells were passaged with trypsin/EDTA to maintain subconfluency.

To study the effects of mitotic inhibitors on asynchronous cultures, cells were seeded into 60-mm dishes and incubated 24–48 hr. Concentrated stock solutions of Colcemid (GIBCO), nocodazole (Sigma), and taxol (provided by the National Cancer Institute), were added directly to cultures which were <50% confluent to achieve the specified final concentrations. Cells were harvested by washing once with phosphate-buffered saline (PBS; GIBCO), detached with EDTA/trypsin, and fixed in 70% (vol/vol) ethanol at the specified times after drug addition.

For flow cytometric analysis and calculation of mitotic index, samples were incubated in PBS containing RNase A at 500 μg/ml and propidium iodide at 5 μg/ml, overnight at 4°C. The mitotic index was calculated by scoring a minimum of 200 cells.

Cells were synchronized by mitotic selection without addition of mitotic inhibitors, using a previously described method (6). In studies of drug effects in synchronized populations, drugs were added 4 hr after mitotic selection and cells were maintained continuously in drug thereafter.

Preparation of Cell Extracts. Cultures were trypsinized and centrifuged at 150 × *g* for 7 min. Cell pellets were washed once with 10 ml of cold PBS, then resuspended in 1 ml of cold PBS, transferred into a microcentrifuge tube, and pelleted again at 1000 × *g* for 4 min. The cell pellet was frozen in liquid nitrogen and stored at −70°C.

Samples were thawed on ice for 10 min, resuspended in a 100 mM potassium phosphate buffer, pH 7.0, and lysed by rapid freezing and thawing (two cycles). Lysates were then sonicated in a cup-horn sonicator for three rounds of 10 sec while maintained in an ice-water bath and centrifuged at 12,000 × *g* for 15 min at 4°C. The total protein concentration of each lysate was determined by Bradford assay (Bio-Rad).

H1 Kinase Activity Assay. Equivalent amounts of protein from each sample (usually 10 μg) were used for each kinase reaction. The reactions were carried out in 50 μl total volume of 50 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/1 mM dithiothreitol/50 μM ATP, containing purified histone H1 (Boehringer Mannheim) at 50 μg/ml and 10 μCi of [γ-^{32}P]ATP (>3000 Ci/mmol, Amersham; 1 Ci = 37 GBq). The reaction mixtures were incubated at 30°C for 30 min, and the reactions were stopped by the addition of 50 μl of 2× sample buffer [0.125 M Tris/20% (wt/vol) glycerol/10% (vol/vol) 2-mercaptoethanol/4.6% (wt/vol) SDS]. Samples were boiled 3 min, and then 50 μl of each sample was electrophoresed on an SDS/12% polyacrylamide gel according to the method of Laemmli (7), fixed, and stained with 0.1% Coomassie blue in

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*To whom reprint requests should be addressed.

50% trichloroacetic acid to confirm the uniformity of protein loading, and analyzed by autoradiography. Histone H1 labeling was quantitated by a scanning laser densitometer (LKB).

Immunoblotting. An equivalent amount of total protein from each lysate was precipitated in acetone at −20°C. The precipitates were centrifuged at 12,000 × *g* for 15 min at 4°C and resuspended in 50 μl of sample buffer. Following SDS/PAGE, samples were transferred to nitrocellulose membrane (0.45 μm pore size, Schleicher & Schuell) by semidry electrotransfer (LKB) at 0.8 mA/cm^2, for 2–2.5 hr. The filter was stained with 0.2% Ponceau S in 5% trichloroacetic acid to confirm the uniformity of protein loaded. The filter was then washed three times with ≈200 ml of Tris-buffered saline (TBS; 10 mM Tris·HCl, pH 7.4/150 mM NaCl/5 mM $MgCl_2$), and blocked with 3% gelatin in TBS at room temperature for 2 hr. Subsequently, the filter was washed twice with TBS and incubated overnight at room temperature with primary antibody, either anti-human cyclin B (gift of Jonathan Pines, Salk Institute) or anti-human p34^{cdc2} (gift of Paul Nurse, University of Oxford), diluted 1:500 in TBS/1% gelatin. The filter was washed four times with ≈200 ml of TBS and then incubated 1 hr at room temperature with the secondary alkaline phosphatase-conjugated antibody (Sigma) diluted 1:1000 in TBS/1% gelatin. Following four washes with TBS, alkaline phosphatase activity was determined by a nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) color development system (Bio-Rad).

Immunoprecipitation. For immunoprecipitation, 60-mm dishes of cells at ≈75% confluency were harvested and lysed in 100 mM potassium phosphate buffer, as described above. Subsequent immunoprecipitations and H1 kinase reactions were performed in the presence of the following mixture of protease inhibitors: aprotinin at 5 μg/ml, leupeptin at 5 μg/ml, phenylmethylsulfonyl fluoride at 1 mM, tosyllysyl chloromethyl ketone at 100 μg/ml, and tosylphenylalanyl chloromethyl ketone at 50 μg/ml. Antiserum recognizing the carboxyl terminus of the human p34^{cdc2} was added (10 μl) to the lysate for a final volume of 220 μl, and the mixture was incubated 1 hr on ice. Immune complexes were immobilized with 10 μl of staphylococcal protein A-Sepharose (Sigma). The mixture was centrifuged, and the supernatant was removed and incubated again with 10 μl of protein A-Sepharose. The two protein A-Sepharose immune complex pellets were pooled, washed three times with 1 ml of 100 mM potassium phosphate buffer, and then resuspended in buffer to a final volume equivalent to the initial immunoprecipitation volume (220 μl). Equal volumes of supernatant and resuspended complexes were then assayed for H1 kinase activity, as described above.

RESULTS

Cell Line-Specific Effect of Mitotic Inhibition. Flow cytometric analysis of asynchronous cultures treated with various concentrations of Colcemid revealed cell line-specific differences in the ability of the cells to progress into a second S phase in the absence of mitosis, measured as the percentage of cells with >4C DNA content (C = haploid DNA content). At one extreme, CHO-AA8 cells progressed to 8C and subsequently 16C DNA content over the course of 72 hr of treatment with Colcemid (Fig. 1 *B* and *C*). During the course of prolonged treatment, these cells assumed a refractile mitotic morphology, but eventually they resumed an interphase morphology without dividing and became micro- and multinucleated. At the other extreme, HeLa S3 cells progressed to the 4C (G_2/M) position and remained blocked there throughout 72 hr of Colcemid treatment (Fig. 1 *A* and *C*). Microscopically, the cells retained a refractile mitotic morphology throughout the course of treatment. The broadening of the 4C peak observed in the 48- and 72-hr histograms reflects a loss of fluorescence associated with cell death (unpublished results). Cytologic examination indicated that the cells maintained condensed chromatin without reformation of a nuclear membrane, and hence were arrested in mitosis rather than a polyploid G_1 state.

The effect of prolonged Colcemid treatment in different mammalian cell lines varied significantly (Table 1). The percentage of cells with >4C DNA content determined after 72 hr of Colcemid treatment was found to vary between essentially 0% and 100%, depending upon cell line. Such differences were not consistently correlated with population doubling times, and therefore were not due solely to cell line differences in the rate of cell cycle progression. Similar effects were observed over a wide range of Colcemid concentrations (7–700 μg/ml), as well as with nocodazole (1.5 μM) and taxol (1–10 μg/ml). In all cases, concentrations sufficient to prevent cell division gave similar results. The effects at all concentrations of the three drugs tested were limited to mitosis, without any appreciable effects on progression through other cell cycle phases, as determined by flow cytometric analysis. We therefore conclude that the observed cell line-specific differences in cell cycle progression in the absence of mitosis were due to differences in response to disruption of the mitotic apparatus and not due to differences in cell killing, drug uptake, or other cell type-specific drug effects.

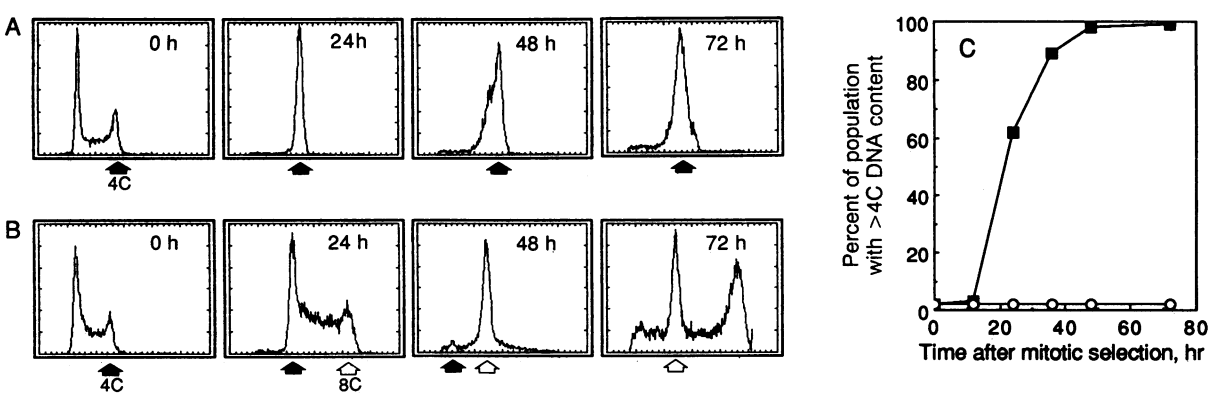

FIG. 1. Effect of Colcemid on asynchronous HeLa S3 and CHO-AA8 cells. Colcemid was added to asynchronous cultures of HeLa S3 (*A*) and CHO-AA8 (*B*) cells to a final concentration of 70 ng/ml. At 0, 24, 48, and 72 hr after drug addition, cells were harvested, fixed, stained with propidium iodide, and subsequently analyzed for DNA content by flow cytometry, with frequency plotted as a function of DNA content. Arrows indicate position of cells with 4C (▲) and 8C (△) DNA content. (*C*) Percentage of cells with >4C DNA content over the course of 72 hr of Colcemid treatment for cultures of HeLa S3 (○) and CHO-AA8 (■) cells.

Table 1. Cell line differences in Colcemid response

Cell line	Description*	PDT, hr	% >4C
CHO-AA8	Chinese hamster ovary; α-MEM	13	98
CHO-K1	Chinese hamster ovary; α-MEM	18	95
SV-T2	SV40-tr. 3T3, tumorigenic; DMEM	14	82
BALB/3T3	Mouse immortalized; DMEM	17	59
3T6	Mouse immortalized; DMEM	14	58
293	Human Ad5-tr. kidney; DMEM	21	42
Vero	Vervet kidney; α-MEM	27	11
—	Primary mouse embryo; DMEM	21	10
MES-SA	Human uterine sarcoma; RPMI	ND	8
Tera2	Human germ cell tumor; DMEM	ND	8
SV-IMR	SV40-tr. IMR-90; F-12/α-MEM	20	6
IMR-90	Human limited life span; F-12/α-MEM	20	5
DHL-4	Human histiocytic leukemia; RPMI	ND	2
TAB	Human Burkitt lymphoma; RPMI	ND	2
HeLa S3	Human adenocarcinoma; α-MEM	23	2

Asynchronous cultures at <50% confluency at the time of drug addition were treated for 72 hr with Colcemid at 70 ng/ml and the percentage of cells with DNA content >4C was determined by flow cytometry. Population doubling time (PDT) was measured in logarithmic-phase cultures. ND, no data.

*Cells were transformed (tr.) by simian virus 40 (SV40) or adenovirus 5 (Ad5) where indicated. The primary mouse embryo cells were isolated from 10- to 12-day-old embryos. All media were supplemented with 10% fetal bovine serum, gentamicin at 50 μg/ml, and 2 mM glutamine, buffered with 10 mM Hepes at pH 7.4. Cells were incubated at 37°C. α-MEM, minimal essential medium α/5% CO_2; DMEM, Dulbecco's modified Eagle's medium/10% CO_2; RPMI, RPMI medium 1640/5% CO_2; F-12/α-MEM, 1:1 Ham's F-12 medium and minimal essential medium α/5% CO_2.

Colcemid and Nocodazole Effects on Cyclin B and $p34^{cdc2}$. Cultures of CHO-AA8 and HeLa S3 cells were synchronized by detachment of mitotic cells. After mitotic selection, cultures were incubated 4 hr to allow mitotic cells to progress to G_1, at which time Colcemid was added for a final concentration of 70 ng/ml. Immunoblot analyses were performed and histone H1 kinase activity was determined at the specific times after selection. Immunoblots were made with either an antiserum raised to human cyclin B (8) or an antiserum raised to the carboxyl terminus of human $p34^{cdc2}$ (9). In both HeLa S3 and CHO-AA8 cells complete disruption of spindle assembly was verified by anti-tubulin immunofluorescence.

CHO-AA8 cells progressed into S phase by 8 hr and accumulated at the 4C (euploid G_2/M) position 12 hr after mitotic selection (Fig. 2*A*). In these cells, cyclin B levels (Fig. 3*A*) and histone H1 kinase activity (Fig. 3 *C* and *D*) increased significantly at 12 hr and peaked 16 hr after mitotic selection. Both cyclin B levels and histone H1 kinase activity decreased to G_1 levels after 24 hr as cells exited (without dividing) the euploid M phase. The cells entered a subsequent S phase after 30 hr and transiently accumulated at the 8C (polyploid G_2/M) position 36 hr after mitotic selection (Fig. 2*A*). Both cyclin B (Fig. 3*A*) and histone H1 kinase activity (Fig. 3 *C* and *D*) increased again as the cells entered the polyploid M phase (8C). These results indicate that the cyclic rise and fall of cyclin B and histone H1 activity persists in the absence of mitosis in CHO-AA8 cells. Furthermore, cellular levels of $p34^{cdc2}$ did not vary significantly throughout the course of treatment (Fig. 3*B*).

HeLa S3 cells progressed into S phase 12 hr after mitotic selection, accumulated in the 4C position after 18 hr, and remained blocked in that position up to 48 hr after mitotic selection. These cells did not undergo chromatin decondensation and eventually became pyknotic. Cyclin B levels (Fig. 3*A*) and histone H1 kinase activity (Fig. 3 *C* and *D*) increased to high levels by 18 hr and remained elevated throughout the course of mitotic block, indicating that the destruction of cyclin B and inactivation of histone H1 kinase activity failed to occur in the absence of mitosis. Again, $p34^{cdc2}$ levels were invariant (Fig. 3*B*). Thus, these cells remain arrested in a

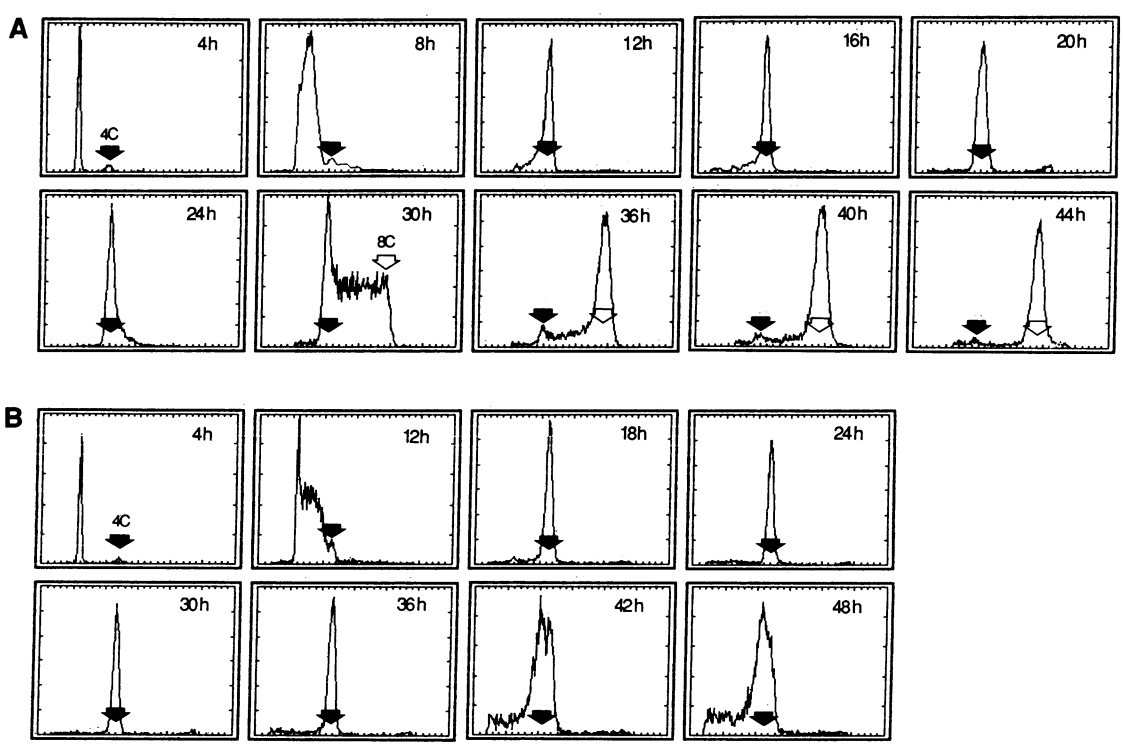

FIG. 2. Cell cycle progression of synchronized cultures of CHO-AA8 (*A*) and HeLa S3 (*B*) cells. Cells were synchronized by mitotic selection and Colcemid was added to the cultures 4 hr after mitotic selection to a final concentration of 70 ng/ml. Cells were harvested at the specified times after mitotic selection, and aliquots were prepared for flow cytometric analysis and measurements of histone H1 kinase activity and cyclin B levels.

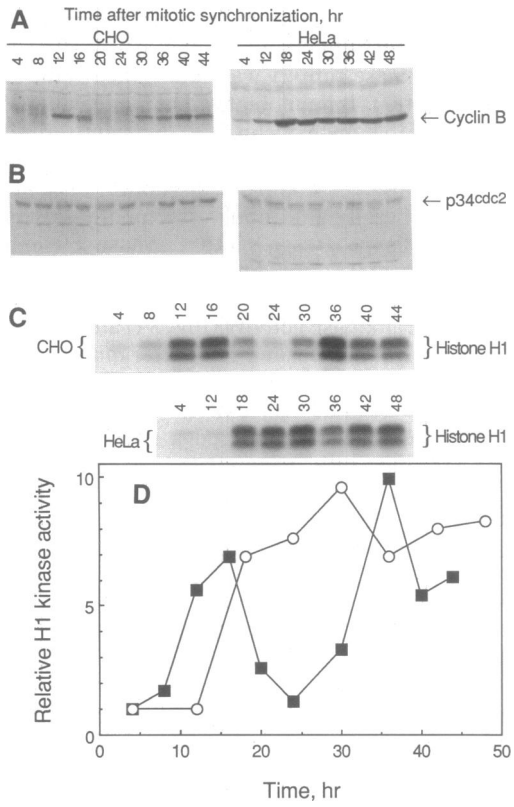

FIG. 3. Cell cycle variations in cyclin B levels and H1 kinase activity. Whole cell lysates were prepared from synchronized cultures treated with Colcemid as described for Fig. 2. (*A*) Cellular levels of cyclin B were determined by immunoblot with an antiserum to human cyclin B. Equal amounts of total protein were loaded from each sample. (*B*) Immunoblot of $p34^{cdc2}$ with an antiserum recognizing the carboxyl terminus of the human $p34^{cdc2}$. (*C*) Histone H1 kinase activity was assayed for equal amounts of total protein from each lysate sample. (*D*) Densitometric quantitation of histone H1 kinase activity in *C*. Activities are expressed relative to the kinase activity at 4 hr after mitotic selection. ■, CHO-AA8; ○, HeLa S3.

mitotic state in response to disruption of the mitotic spindle apparatus.

Identical results, as determined by flow cytometry and histone H1 kinase assays, were obtained with nocodazole (data not shown). Specifically, in the presence of 1.5 μM nocodazole, HeLa S3 cells progressed to and remained arrested in mitosis, with elevated levels of histone H1 kinase activity, throughout the course of treatment. CHO-AA8 cells were only transiently delayed in progressing into the next cell cycle, with coincident changes in histone H1 kinase activity. Again, in both cell types, complete disruption of spindle assembly was verified by anti-tubulin immunofluorescence.

Immunoprecipitation of $p34^{cdc2}$. To preclude the possibility that the measured histone H1 kinase activity was due in part to nonspecific kinase activity, a lysate prepared from HeLa cells blocked 18 hr with Colcemid was immunoprecipitated with an antiserum against the HeLa $p34^{cdc2}$ carboxyl terminus. At an equivalent final dilution, all histone H1 kinase activity segregated with the immunoprecipitated fraction (Fig. 4, lane B), with no detectable activity present in the supernatant (Fig. 4, lane A). On the basis of these results, we conclude that the histone H1 kinase activity measured in whole cell lysates is an accurate and quantitative measure of $p34^{cdc2}$ activity.

Colcemid-Induced Mitotic Delay in CHO-AA8 Cells. To determine the effect of Colcemid on the duration of mitosis in CHO-AA8 cells, histone H1 kinase activity and the mitotic index were measured at the specified times after mitotic

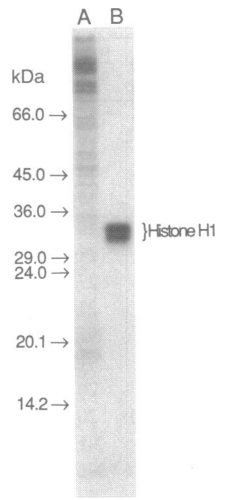

FIG. 4. Immunoprecipitation of mitotically blocked HeLa S3 extract. A lysate prepared from a HeLa S3 culture treated 18 hr with Colcemid at 70 ng/ml was immunoprecipitated with an antiserum to human $p34^{cdc2}$. Equal volumes of the supernatant (lane A) and the resuspended immunoprecipitate (lane B) were assayed for histone H1 kinase activity.

synchronization, in the presence or absence of Colcemid at 70 ng/ml (Fig. 5). In general, changes in histone H1 kinase activity paralleled changes in the mitotic index (Fig. 5 *B* and *C*). In the absence of Colcemid, the mitotic index and histone H1 kinase activity peaked 14 hr after mitotic selection, then declined the following 6 hr, reaching a minimum at 20 hr, and subsequently increased again, beginning 24 hr after mitotic selection (Fig. 5 *A* and *B*). These results are consistent with the previously calculated population doubling time of 13 hr (Table 1). A characteristic rapid decay in synchrony is probably responsible for the observation that histone H1 activity does not drop to G_1 (4-hr) levels 20 hr after selection.

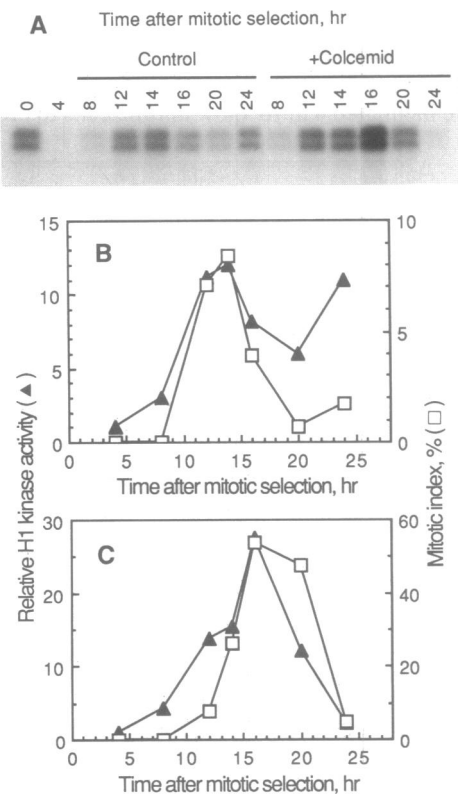

FIG. 5. Colcemid-induced delay of mitotic traversal in CHO-AA8 cells. Cells were synchronized by mitotic selection and Colcemid was added at 70 ng/ml to one set of cultures 4 hr after synchronization. (*A*) Histone H1 kinase activity assayed for equal amounts of total protein. Densitometric quantitation of *A* closely paralleled the mitotic indices calculated for cultures either in the absence of (*B*) or in the presence of (*C*) Colcemid. Histone H1 kinase activities are expressed relative to the activity at 4 hr after synchronization.

In the presence of Colcemid, histone H1 activity and the mitotic index peaked at 16 hr and decreased to near G_1 levels 24 hr after mitotic selection (Fig. 5*C*). Inasmuch as the onsets of increase in H1 kinase activity and mitotic index were nearly identical (Fig. 5 *B* and *C*), we conclude that the entry into mitosis was not significantly delayed by the presence of Colcemid. These results indicate that Colcemid treatment results in a transient 2- to 4-hr delay in the exit from mitosis in this cell line.

DISCUSSION

We have examined the effects of prolonged inhibition of mitosis with agents which disrupt microtubule polymerization in different mammalian cell lines, and we have found that significant differences exist between cell lines in their ability to progress into subsequent cell cycles in the absence of mitosis. Such differences appeared to be, in part, species related. Thus, all the human cell lines examined, with the exception of 293 cells, remained permanently blocked in a mitotic state in the presence of agents which inhibit the assembly of the mitotic spindle apparatus. Vero cells (African green monkey) were also "human-like" in their response to Colcemid. Rodent cell lines, on the other hand, were generally able to progress into subsequent cell cycles in the absence of mitosis, resulting in serial doublings of DNA content. Furthermore, in mouse cells, there was an apparent correlation between an increasingly transformed phenotype and an increased propensity to become polyploid such that primary mouse embryo cultures remained blocked in mitosis, fully transformed cultures (SV-T2) readily became polyploid, and spontaneously immortalized lines (3T3 and 3T6) had an intermediate response to Colcemid treatment.

The activation of $p34^{cdc2}$, which involves an interaction with cyclin, is a biochemical process central to the induction of mitosis and is directly or indirectly responsible for the induction of nuclear membrane breakdown, chromosome condensation, spindle assembly, and cyclin degradation (1, 2). The exit from mitosis and progression into interphase is thought to require the degradation of cyclin and the inactivation of $p34^{cdc2}$ (2). We examined $p34^{cdc2}$ kinase activity and cyclin B levels in HeLa S3 and CHO-AA8 cells in the presence of Colcemid and found that these biochemical parameters of mitosis varied in concordance with cell behavior as determined by flow cytometry and microscopic examination. Thus, in HeLa cells, disruption of spindle assembly was correlated with maintenance of elevated cyclin B levels and continued activation of $p34^{cdc2}$ (measured as histone H1 activity). These results are similar to the findings of Pines and Hunter (8), who reported that HeLa cells treated 12 hr with nocodazole had elevated levels of cyclin B and $p34^{cdc2}$ activity. This sustained mitotic arrest could result either from the continual synthesis of cyclin B and activation of $p34^{cdc2}$ or as a result of preventing the degradation of cyclin B. Preliminary experiments in which cycloheximide was added to mitotically arrested HeLa cells suggest that maintanance of the mitotically arrested state is not dependent on continued protein synthesis. This suggests that in these cells cyclin B degradation is prevented even while $p34^{cdc2}$ persists in an activated state.

In contrast, the periodic oscillation of cyclin B levels and $p34^{cdc2}$ activity was not inhibited by the disruption of microtubule assembly in CHO-AA8 cells. Exit from mitosis was transiently delayed (2–4 hr) in the absence of a mitotic spindle apparatus, with cyclin B levels and $p34^{cdc2}$ activity declining to interphase levels as cells exited from mitosis without dividing. Cyclin B levels and $p34^{cdc2}$ activity increased again as cells progressed into M phase of the subsequent cell cycle. These results indicate that progression into the subsequent cell cycle and initiation of DNA synthesis are not dependent on spindle assembly, karyokinesis, or cytokinesis in this cell type.

These cell line-specific differences were reproducible at all concentrations of Colcemid, nocodazole, or taxol sufficient to completely disrupt spindle assembly. Similarly, temperature-sensitive CHO cell lines with mutated α- or β-tubulins have been isolated that, at the nonpermissive temperature, have phenotypes identical to that reported herein (10). Such mutants have an increased duration of mitosis, are defective for cytokinesis, and become micro- and multinucleated, but continue to cycle at nonpermissive temperatures. These results indicate that the observed cell line differences are dependent only upon the disruption of spindle assembly and are not simply the result of the pharmacological effects of the agents used.

It is becoming increasingly apparent that the cell cycle is controlled by complex biochemical interactions and that the proper temporal order of cell cycle events is maintained by mechanisms—i.e., checkpoints—which serve to couple the initiation of later events with the completion of earlier events (4). Some early embryonic cells appear to have fewer checkpoints than somatic cells. Murray and Kirschner (2) have suggested that a checkpoint exists that couples exit from mitosis with proper spindle assembly. Our results support the existence of such a checkpoint in human/primate cell lines in which the absence of a functional spindle apparatus leads to complete arrest in mitosis. In rodent cell lines, however, either such a checkpoint does not exist or the stringency of control is much lower than in human cells. The finding that in mouse cells increasingly transformed phenotype is correlated with increased propensity for progressing into subsequent cell cycles in the absence of mitosis suggests that such a checkpoint does exist, but that the loss of stringency of control may represent one aspect of immortalization and/or tumorigenicity. The existence of tumor suppressors and the loss of growth control consequent to their disruption indicate that the loss of normally negative acting proliferation controls can be involved in cellular transformation (11).

Aside from the differences which exist between embryonic and somatic cells, the biochemical pathways which control mitosis are thought to be universally conserved in all eukaryotic cells (1). The basic conclusion of this report, however, is that clear differences exist between mammalian cell lines in the mechanisms controlling the exit from mitosis and progression into a subsequent cell cycle. Such differences may have important implications for the propensity for gene amplification and susceptibility to various cytotoxic agents, as will be reported elsewhere.

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1. Nurse, P. (1990) *Nature (London)* **344,** 503–508.
2. Murray, A. W. & Kirschner, M. W. (1989) *Science* **246,** 614–621.
3. Lewin, B. (1990) *Cell* **61,** 743–752.
4. Hartwell, L. H. & Weinert, T. A. (1989) *Science* **246,** 629–634.
5. Enoch, T. & Nurse, P. (1990) *Cell* **60,** 665–673.
6. Mariani, B. D., Slate, D. L. & Schimke, R. T. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4985–4989.
7. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
8. Pines, J. & Hunter, T. (1989) *Cell* **58,** 833–846.
9. Bailly, E., Dorée, M., Nurse, P. & Bornens, M. (1989) *EMBO J.* **8,** 3985–3995.
10. Abraham, I., Marcus, M., Cabral, F. & Gottesman, M. M. (1983) *J. Cell Biol.* **97,** 1055–1061.
11. Stanbridge, E. J. (1990) *Science* **247,** 12–13.