Differential Effect of Ionizing Radiation on the Expression of Cyclin A and Cyclin B in Hela Cells

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ABSTRACT

Ionizing radiation induces a G2 delay in eukaryotic cells. Since mitotic cyclins are required to trigger the transition from G2 into and through mitosis, we chose to investigate their expression after irradiation in Hela cells. In normally cycling Hela cells, both cyclin A and B mRNA and protein levels rise dramatically in G2/M and rapidly fall coincident with the completion of mitosis. The rise of cyclin A mRNA at the S/G2 boundary slightly precedes that of cyclin B mRNA. Although the peaks of expression of each of these molecules overlap, cyclin A mRNA and protein diminish before cyclin B. After irradiation in S, cyclin A mRNA and protein levels rose with the same kinetics as in the controls, but ultimately exceeded the levels seen in the control population. Cyclin A mRNA and protein levels remained high throughout the G2 delay induced by irradiation. In contrast, cyclin B mRNA and protein levels did not rise as the irradiated cells entered G2/M. Only later, before the irradiated cells exited from G2/M, did levels of cyclin B reach the levels seen in the unirradiated controls. The decreased amount of cyclin B mRNA and protein was inversely proportional to the dose of radiation. These data indicate that irradiation that results in a G2 delay appears to block cells at a point after production of cyclin A but before cyclin B can be fully expressed and that cells do not exit from the delay until cyclin B is again expressed. Thus, cyclin A and cyclin B expression respond differentially to radiation, with cyclin A rising at the same time as the control and to even higher levels than seen in the controls, whereas cyclin B shows a temporal delay in expression.

INTRODUCTION

A universal effect of ionizing radiation on growing eukaryotic cells is induction of a division delay. The most characteristic of these effects is the prolongation of the G2 phase of the cell cycle (1-8). This effect on G2 phase is also seen after exposure of cells to some chemotherapeutic drugs and other DNA-damaging agents (9-11). There is some evidence that links these perturbations in G2 phase to the sensitivity of the cells to the killing effects of ionizing radiation. The mutant, rad9, of Saccharomyces cerevisiae, which is highly sensitive to radiation, is also characterized by an absence of a radiation-induced G2 delay (12). Conversely, in the system of oncogene-transfected primary rat embryo cells, which we have developed for studying radiation resistance in H-ras-transfected rat embryo cell lines, the radiosensitive phenotype is associated with a prolongation in the radiation-induced G2 delay in these same cell lines (13, 14).

In recent years, a number of the biochemical events that appear to trigger mitosis have been identified. Much of the work has been done in yeast, both Saccharomyces pombe and S. cerevisiae, and in oocytes of various species, but a number of the identified mechanisms also apply to mammalian cells (15, 16). In all of these systems, the levels of proteins called cyclins rise in late S or G2 before mitosis is initiated, and then fall after mitosis has been completed (17-19). The linkage of this rise to the initiation of mitosis is based upon the following experimental evidence. (a) Injection of surf clam cyclin A mRNA into resting Xenopus oocytes triggered their meiotic M phase (20). (b) Blockage of cyclin synthesis can block mitosis (21). (c) Fission yeast with a conditional lethal mutation in a gene homologous to the cyclins of higher organisms is blocked in cell division (22, 23). This evidence together indicates that the mitotic cyclins are necessary for the triggering of mitosis.

In human cells, 2 mitotic cyclins, cyclin A and B, have been identified based on their homology to sea urchin cyclins A and B (16). Cyclin mRNA levels rise in Hela cells at late S phase and peak in G2. Unlike oocytes, in which cyclin levels are regulated solely through translational controls, levels of mitotic cyclins appear to be regulated in Hela cells through mRNA levels as well as at the protein level. There are differences in the behavior of the 2 cyclins in Hela cells. The protein for cyclin A begins to accumulate earlier and also declines at an earlier point in the cycle in Hela cells and in Xenopus oocytes (24, 25). Thus, cells blocked in metaphase in mitosis by the drug nocodazole have high levels of cyclin B but baseline levels of cyclin A (18, 25).

The cyclins mediate their action through a complex with p34cdc2 kinase, which is highly active as a histone H1 kinase and whose activity peaks in M phase (15, 16). Mitosis in oocytes as well as a wide variety of other cells can be triggered by maturation promoting factor, which was first identified because injection of the contents of an egg about to undergo mitosis into a resting oocyte would trigger mitosis. This material has been highly purified, and its activity is associated with 2 proteins, one a cyclin and the other p34cdc2 kinase (15, 16). Thus, the suggestion is that part of the signal triggering mitosis involves a rise in cyclin levels, and that this cyclin then binds to p34cdc2 kinase, altering the activity of the kinase. In addition, the activity of p34cdc2 kinase is regulated by a series of phosphorylation and dephosphorylation events events (26-33). Degradation of cyclin is required to complete mitosis (34, 35). In Hela cells, cyclin B complexes with p34cdc2, whereas cyclin A is also found in a complex with a different molecule that shares some antigenic determinants with p34cdc2 (18). The actual role of both of the cyclins in human cells is not yet defined, but in Drosophila it has been demonstrated that cyclins A and B are both required for completion of the cell cycle (36). In oocytes depleted of cyclin A, mitosis is uncoupled from DNA synthesis, suggesting a role for cyclin A as a regulatory element (37). It is not known at this time whether one cyclin is required to activate the complexes formed by the other in a sequential fashion or if both are activated and are both independently required.

Lock and Ross (38) have shown that radiation with X-rays or treatment with etoposide leads to substantially decreased p34cdc2 H1 kinase activity. In an attempt to understand the phenomena of G2 delay after radiation, we studied the effects of X-ray exposure on cyclin B expression, both the mRNA and the protein, in Hela cells (39). Two effects were seen after exposure of synchronized cells to doses of X-rays ranging from 6 to 10 Gy. If the cells were exposed to radiation in the S phase of the cell cycle, a delay was seen in the expression of the mRNA for the cyclin B gene. If however, the cells were exposed to X-rays in the G2 phase of the cell cycle, when mRNA levels were already rising, the effect on mRNA expression was much smaller, but a significant delay was seen before cyclin B protein levels started to...
rise. The results imply that ionizing radiation can effect a delay in cyclin B expression at both the mRNA and protein levels, depending on the phase of the cell cycle at which cells are exposed.

In this study, we extend these experiments to show that the effect of radiation on cyclin B expression is seen not only after exposure to high doses of X-rays, but also at the lower levels of radiation, which are used in the treatment of cancer in the clinical setting. Furthermore, we show that the effect of delaying expression seems to be specific to cyclin B, since initiation of cyclin A expression is not delayed by radiation. Indeed, cyclin A accumulation reaches even higher than normal levels during the radiation-induced G2 delay. These data allow us to better focus on the points within G2 at which radiation may exert its effects.

MATERIALS AND METHODS

Synchronization of Hela Cells. Hela cells (1 x 10⁶) were plated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum on 100-mm tissue culture dishes and cultured at 37°C in a CO₂ incubator. The cells were synchronized using a modification of the procedure developed by Heintz et al. (40). Two days after plating, 2 μg thymidine was added to the medium for 12 h. It was washed out and the cells were cultured for another 18 h in the presence of 20 μM thymidine and deoxyctydine. Then 5 mg/ml aphidicolin was added for 12 h and washed out. Thirty min after release from the aphidicolin, the experimental cells were exposed to the indicated doses of X-rays using a Siemens therapeutic X-ray machine operated at 250 kV, 15 mA with a 2-mm Al filter (effective energy. 70 kV). The control cells were mock-irradiated for the same time. Immediately after irradiation and at various times thereafter, cells were harvested for DNA content analysis, for extraction of RNA, and for lysates to be used for immunoblotting. Distribution of cells through the cell cycle was measured in each experiment by flow cytometry after fixation of the cells in 70% ethanol. The methods used were as described by Gohde et al. (41), and calculation of the percentage of cells in each phase of the cell cycle was performed using software supplied by Partek.

Preparation of Polyclonal Antibody to Human Cyclin B. Based on an analysis of the sequence of the human cyclin B as published by Pines and Hunter (25) in comparison to the sequences of the rat (Rattus norvegicus) and Chinese hamster cyclin B cDNAs from our own laboratory, we chose to raise an antibody against a region that diverged significantly between the species. The sequence used was KKEAKPSATGKVIDKKKL. An analysis of its hydrophilicity suggested it was likely to lie on the surface of the molecule, and the human sequence appeared to contain a 6-amino acid insert in this region that was not found in either of the rodent species, suggesting that this sequence was likely to be immunogenic in a rabbit. This peptide was synthesized with a C-terminal cysteine by the Protein Chemistry Laboratory at the University of Pennsylvania and coupled to maleimide-activated keyhole limpet hemocyanin (Pierce, Rockford, Ill.). Antisera to cyclin A were a gift from Dr. Pines and Hunter. Filters were exposed at −70°C to Kodak X-AR film.

Immunoblotting. Immunoblots were performed on samples derived from 5 x 10⁶ cells as described by Pines and Hunter (18, 25). Twenty μg of total cellular protein were loaded into each lane. Antisera to cyclin B were the antibody to the synthetic peptide as described above and used at dilutions of 1:500. A LKB laser scanning densitometer equipped with Gelscan software was used to quantify the intensity of the cyclin bands on the autoradiographs above. The linearity of the response of this antibody was determined by electrophoresing different amounts of a Hela cell extract from Hela cells blocked in G2/M for 24 h with 0.4 μg/ml (Sigma) nocodazole. The resultant gel is shown in Fig. 1A, and the plot of the densitometry scan of that gel in Fig. 1B. Thus, this antibody used in the conditions of these experiments will provide a linear response. Antisera to cyclin A were a gift from Dr. Pines and Dr. Hunter. Filters were exposed at −70°C to Kodak X-AR film.

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3. A. Markiewicz et al., unpublished observations.
4. The abbreviation used is: SSC, standard saline-citrate.
RESULTS

Dose Response of Cyclin A and B to X-Rays. To determine the dose response of mitotic cyclin levels to X-rays, HeLa cells were irradiated in S and then harvested in G2 for flow cytometry, mRNA, and protein analysis. HeLa cells were synchronized using an S phase block with thymidine followed by a block with aphidicolin as described in "Materials and Methods." Three h after release of the aphidicolin block, when the majority or the cells had entered S phase, the cells were irradiated with various doses of X-rays. To monitor the effects of X-rays on cell cycle progression, the position of the cells in the cycle was monitored using flow cytometry for DNA content 8, 10, and 12 h after irradiation. Fig. 1A shows the cell cycle progression at 8 and 10 h after the initial release from aphidicolin after irradiation in S phase with doses from 2 to 12 Gy. By 8 h, 80% of the control, mock irradiated, cells were in G2/M phase. At 10 h, the G2 delay induced by irradiation is apparent in that from 70 to 90% of the cells that had received from 2 to 12 Gy remained in G2, whereas 80% of the control cells had re-entered G1 at that time (Fig. 2B). The length of the G2 delay induced by radiation is dose dependent (1-8). Thus, by 12 h, the cells that had received the lowest dose, 2 Gy, had exited from G2/M with 70% of the cells in G1 at that time, whereas the cells that had received higher doses from 6–12 Gy still remained in G2/M. At the lower doses, 2 and 4 Gy, although the G2 delay is shorter, it is still evident at 10 h with 70 and 85% of the irradiated cells in G2/M phase, whereas the controls had exited into G1 at that time. Regardless of the length of the delay induced, cells given from 2–12 Gy in S were in an X-ray-induced G2 delay at 10 h after release from the block in this experiment.

Although the division delay induced by radiation is predominantly in G2 phase, some cells, including HeLa cells, do undergo smaller delays in S phase (44). This feature can be seen in this experiment. At 8 h, the cells given the higher doses from 6 to 12 Gy remained in S, although the controls and the cells given lower doses are in G2/M (Fig. 2). However, by 10 h the majority of the irradiated populations had entered G2. Thus, we chose to compare cyclin levels at 10 h in the irradiated samples when most of the irradiated cells were in G2/M.

Cyclin A mRNA levels and protein levels were measured on RNA blots and immunoblots from the samples obtained after irradiation in S, and then collected at 8, 10, and 12 h after irradiation. These data are shown for the time points at 8 and 10 h after release in Fig. 3. At 8 h, the unirradiated sample had a readily detectable level of cyclin A mRNA. The cells that had been irradiated with from 2 to 10 Gy had virtually identical amounts of cyclin A mRNA. Cyclin A mRNA levels from the 10-h time point, when all of the samples except the unirradiated controls were in G2/M, is plotted against dose of X-rays in Fig. 3A. These data indicate that there is no decrease in cyclin A mRNA levels with increasing doses of X-rays.
levels with doses from 2 to 12 Gy of X-rays at 8 or 10 h. The RNA blot for cyclin A mRNA 10 h after irradiation is shown in Fig. 4. The control for RNA loading is shown in Fig. 5, when the blot was reprobed with a 28S ribosomal RNA probe. Thus, at both 8 and 10 h, the mRNA levels for cyclin A are high and do not decrease with increasing doses of X-rays up to 12 Gy. The cyclin A protein levels as measured on immunoblot are also equivalent at all doses at 8 h, as shown in Fig 3B. At 10 h, the control cells have entered G1, and so at 10 h at 0 dose, the cyclin A protein levels are low. At 2 and 4 Gy, the cyclin A levels somewhat exceed the control values, but at the higher doses the levels of cyclin A are equivalent to those at control values. The immunoblot for the 10-h time point is shown in Fig. 4. Thus, both the cyclin A mRNA and protein levels are essentially unaffected after irradiation in S phase at doses from 2 to 12 Gy, and in fact the cyclin A levels exceed those seen in the control cells at 2 and 4 Gy.

In contrast, cyclin B levels showed a dose-dependent decrease after radiation in S phase as seen in Figs. 5 and 6. Both the mRNA and the protein levels decrease in a fashion inversely proportional to dose. The same samples collected after release from the S block and then irradiated with doses from 2 to 12 Gy of X-rays were subjected to analysis for cyclin B mRNA and cyclin B protein. At 8 h, the amount of cyclin B mRNA and protein is considerably less than at 10 h (data not shown), and again illustrates that cyclin A expression precedes that of cyclin B. Thus, much of the data for cyclin B were evaluated at 10 h. Cyclin B mRNA, but not cyclin B protein, was detected at 10 h after release from S in the unirradiated cells, even though the control cells have passed from G2/M into G1 at that time. This shows that the cyclin B mRNA persists after the protein has been destroyed. We could detect diminished amounts of cyclin B mRNA even at 2 and 4 Gy. At 10 Gy, the cyclin B mRNA level is depressed 4.5-fold (Figs. 5 and 6). However, the levels of 28S ribosomal RNA detected on the RNA blot are essentially the same at all doses, and the cyclin A mRNA levels are also not depressed. The cyclin B protein levels have dropped in the control cells at 10 h as they are in G1, but the protein levels otherwise reflect the decreased amounts of mRNA with decreased amounts of cyclin B being detected on Western blots with the decrease inversely proportional to dose. Thus, our data indicate that cyclin B levels but not cyclin A levels are depressed in a dose-dependent fashion by irradiation.

Effect of X-Rays on Cyclin A and B Protein and mRNA Levels with Time after Irradiation in S Phase. Hela cells were synchronized using a double block of thymidine followed by aphidicolin as described in "Materials and Methods." We irradiated cells at 3 h after release into S with 6 Gy and monitored samples at the indicated times for DNA content and cyclin A and B mRNA and protein levels. In Fig. 7B, the percentage of cells in G2/M after the release from the aphidicolin block is plotted against time. The peak of G2/M was reached at 8 h after irradiation in S in the control population, and the majority of these cells had entered G1 by 10 h. The irradiated population entered G2/M at 8 h, but due to the G2 delay, up to 50% of the population remained in G2/M for up to 16 h after the release from S. The G2 delay does not lead to a delay of exactly the same time in each member of an irradiated population so that cells will exit from the block asynchronously. When cyclin A mRNA levels are compared to the percentage of cells in G2/M from this experiment, it is apparent that the control cyclin A mRNA levels peaked at 8 h, coincident with the G2/M peak (see Figs. 7, 9, and 10). The cyclin A mRNA levels in the irradiated population began to rise at the same time as in the control population, but these levels continued to rise as the cells remain in the block. Cyclin A levels in the irradiated population overshoot the amounts seen in the control population and only fell again as the cells entered G1. Reprobing of this blot with a 28S ribosomal RNA probe indicated that the loading of RNA was equal in all lanes (Figs. 7 and 8). Cyclin A protein levels followed this same pattern, with cyclin A levels rising at the same time as in the unirradiated control population.

![Graphical representation of cyclin A mRNA and protein levels after irradiation in S with varying doses of X-rays.](attached_image)

![Graphical representation of cyclin B mRNA and protein levels after irradiation in S with varying doses of X-rays.](attached_image)
MITOTIC CYCLIN EXPRESSION AFTER HELA IRRADIATION

The effect of irradiation on cyclin B mRNA and protein levels with time. Hela cells were synchronized as described in "Materials and Methods." Cells were irradiated with 6 Gy at 3 h after release from the aphidicolin block, while control cells were taken out of the incubator for the same time. Samples were harvested every 2 h, and processed for DNA content analysis, RNA extraction, and Western blotting. A. densitometric tracing for the RNA blot using a cyclin B probe (top plot), and result of reprobing of the blot used for cyclin B analysis with a 28S ribosomal probe (bottom plot). The relative levels of cyclin B mRNA are plotted against the time after irradiation. O, controls; □, irradiated cells. B. densitometric analysis of the cyclin B protein immunoblots again (top plot) and results of DNA content analysis with the percentage in G2/M plotted against the time after release from the aphidicolin block (bottom plot). O, controls; □, irradiated samples.

Cyclin B mRNA and protein levels showed a different response in irradiated cells. Cyclin B mRNA levels peaked in the control cells, coincident with the peak of cells in G2/M (Figs. 7 and 8). In contrast, the irradiated population showed a delay in the rise of cyclin B mRNA, with the peak levels seen at 10–12 h instead of at 8 h as in the control. The cyclin B protein levels also were lower than that seen in the irradiated cells when a similar percentage of cells was in G2/M (Figs. 7 and 8). The peak of cyclin B occurs at 12 h after radiation as the irradiated population begins to exit G2/M and to enter G1. Thus, cyclin B expression in contrast to cyclin A lags behind the expected amounts seen in the unirradiated cells.

and the cyclin A levels remaining high until 14–16 h after irradiation as the cells exit from G2/M (Figs. 9 and 10). In the control population, cyclin A protein levels peaked at 8 h, coincident with the percentage of cells in G2/M. In the irradiated population, cyclin A levels rose at the same time as the control cells and reached the maximum level seen in the controls at the same time, but continued to increase in amount above the highest levels seen in the controls as the cells remained in G2. The cyclin A levels peaked at 12 h along with the percentage in G2/M and fell as the percentage in G2/M declined. Thus, cyclin A appeared in the G2/M cells at the same time in the controls and the irradiated cells, but continued to rise in the irradiated cells to still higher levels and remained easily detectable after the cyclin A was absent in the control population.

Cyclin C mRNA and protein levels showed a different response in irradiated cells. Cyclin C mRNA levels peaked in the control cells, coincident with the peak of cells in G2/M (Figs. 7 and 8). In contrast, the irradiated population showed a delay in the rise of cyclin C mRNA, with the peak levels seen at 10–12 h instead of at 8 h as in the control. The cyclin C protein levels also were lower than that seen in the irradiated cells when a similar percentage of cells was in G2/M (Figs. 7 and 8). The peak of cyclin C occurs at 12 h after radiation as the irradiated population begins to exit G2/M and to enter G1. Thus, cyclin C expression in contrast to cyclin A lags behind the expected amounts seen in the unirradiated cells.

Cyclin C mRNA

0 Gy 2 4 6 8 10 12 14 16 18 20 22

0 Gy

28S RNA

0 Gy 2 4 6 8 10 12 14 16 18 20 22

6 Gy

Cyclin C Protein

0 Gy 2 4 6 8 10 12 14 16 18 20 22

6 Gy

Fig. 8. Effect of irradiation on cyclin C mRNA and protein levels with time. The samples shown here were collected as described in Fig. 7. Shown is the RNA blot on these samples for cyclin C mRNA, the same blot reprobed with a 28S ribosomal probe and an immunoblot for cyclin C with the time in hours after release from the aphidicolin block indicated above the lane for the sample taken at that time. The controls (0 Gy) are shown above the irradiated samples (6 Gy).
MITOTIC CYCLIN EXPRESSION AFTER HeLa IRRADIATION

Fig. 9. Analysis of the effect of irradiation on cyclin A mRNA and protein levels with time. The samples from the experiment described in Fig. 7 were also analyzed for cyclin A mRNA and protein by reprobing of the RNA blot and immunoblotting with an anticyclin A antisera. Top plot, relative levels of cyclin A mRNA plotted against the time of release of the aphidicolin block; bottom plot, relative cyclin A protein levels as determined by densitometric analysis of the blots shown in Fig. 10. O, controls; •, irradiated samples.

DISCUSSION

The patterns of expression of cyclin A and cyclin B were examined after irradiation in HeLa cells. We found that cyclin B undergoes a dose-dependent decrease in levels of expression in G2 after irradiation in S, whereas cyclin A does not. In following the expression of these 2 cyclins at increasing time after irradiation in S phase with 6 Gy, we noted that cyclin A rose at the same time and with the same kinetics as in the control unirradiated population. With increasing time as the irradiated cells were blocked in G2, the level of cyclin A continued to rise and exceeded that seen in the controls. The level of cyclin A did not decline until the cells exited from G2/M. In contrast when comparing the level of cyclin B to the unirradiated controls, the level in irradiated cells rose only after a delay, not at the same time as the controls, and the levels did not exceed that seen in the controls. Thus, there were coincident peaks in the expression of both cyclins in the irradiated and unirradiated cells, but the pattern of expression of the 2 genes was considerably different.

Radiation produces perturbations in all phases of the cell cycle (1–8). In exponentially growing unirradiated cells, cyclin B message is first seen to rise at or near the S/G2 boundary. In the experiments we report here and in previously reported data, it is evident that irradiation in S phase produces a short S phase delay as well as a longer G2 delay (2). One question that arises from these data is the extent to which the apparent delay in cyclin B expression might be attributable to an S phase delay after irradiation and thus might be unrelated to the subsequent radiation-induced G2 delay. In our previous experiments, we examined the effect of irradiation with 10 Gy during S phase on cyclin B expression in HeLa cells (39). This dose produced a much longer G2 delay than that seen in the experiments we report here. In these previously reported experiments, it was shown that the S phase delay was clearly over and all the cells had progressed into the radiation-induced G2 delay before cyclin B expression rose. Furthermore, the data presented here indicate that when the majority of the cells (65% G2/M, 10% S; Figs. 3 and 7) had progressed past the S/G2 boundary into G2 cyclin B level still remained low. Thus, we conclude that the delay in cyclin B expression has a temporal relationship to the radiation-induced G2 delay and is not a simple consequence of a prolongation of the S phase. The argument is strengthened by noting that cyclin A expression started to rise at the same time in the irradiated and control cells, indicating that the cells had indeed entered G2 since this gene reaches maximal expression in G2/M.

Datta et al. (45) have found similar results in that after irradiation with 5 or 10 Gy of X-rays, cyclin B levels were decreased in U 937 and HL-60 cells. However, at 20 Gy cyclin A, and cdc2 mRNA levels as well as cyclin B RNA levels were depressed through a posttranscriptional mechanism.

The pattern of expression of cyclin A and B that we see in the irradiated cells is consistent with Ruderman's suggestion that it is the appearance of cyclin B that is the signal for the destruction of cyclin A (46). Since cyclin A started to accumulate in both irradiated and unirradiated cells at the same time and since the appearance of cyclin B is delayed in the irradiated population, cyclin A continues to accumulate in the irradiated cells and rises to supernormal levels until cyclin B appears.

These data further indicate that irradiation has some specificity in its effects on both cyclin B mRNA and protein levels, as cyclin A was not so affected. Although some genes have been found to be induced
in cells after irradiation, including members of the heat shock gene family, metallothioneins, c-fos, c-jun, the transcription factor κB, type I collagenase, β-polymerase, and a series of genes called DDI (DNA damage-inducible), which were identified because of their increase in level after DNA damage, others have been shown to be unaffected (47–51). Only cyclin B has been specifically reported to be depressed at the doses considered here. Holland et al. (52) examined the patterns of protein synthesis after irradiation in G2 using one-dimensional gel electrophoresis and found only one band at M, 170,000 to be reduced, the others were not changed. Boothman et al. (53) examined protein synthesis using 2-dimensional gel electrophoresis after irradiation in plateau phase. While the great majority of proteins appeared unaltered, 8 spots increased in intensity and 2 decreased. Thus, the effects on cyclin B mRNA and protein are not a general consequence of irradiation on protein or RNA synthesis and the pattern of their altered expression, while not unique, is unusual.

These data suggest that there is a cellular mechanism that recognizes DNA damage and transduces this signal to result in altered cyclin expression. This mechanism cannot yet be identified because the controls that allow cells to recognize DNA damage and the signals that transduce this recognition are not well understood. The rad9 gene in yeast is one such gene, but its mammalian counterparts have not been identified. Furthermore, it appears to act posttranslationally (54). The RCC1 gene is another potential candidate. tsBN2 cells, which Nishimoto (55, 56) and his group isolated as temperature-sensitive for DNA replication, undergo mitotic events without completion of DNA replication. The wild type gene was cloned by complementation, and tsBN2 proved to have a point mutation in that gene (55, 56). This mammalian gene, which is homologous to the yeast pim-1, functions to regulate p34cdc2 histone H1 kinase activity after the complex of p34cdc2-cyclin B has formed. Thus, it is unlikely to be involved in the events described in this study, but would appear to function through regulation of the sequence of phosphorylation and dephosphorylation events influencing the activity of p34cdc2. The data presented here suggest that one target of the mechanisms regulating G2 transition after DNA damage may involve controls on the levels of cyclin B mRNA.

These data are consistent with but do not prove the idea that the G2 delay in cells that have been irradiated in S may be associated with a time in G2/M after cyclin A has been expressed, but before the initiation of cyclin B expression. There are other G2/M checkpoints for X-ray-induced DNA damage. Tobey (57) identified several points in G2 at which DNA-damaging agents might produce G2 delays. In our previous work, we irradiated cells in G2/M at a time when the mRNA and protein levels for cyclin B were just beginning to rise. Cyclin B mRNA levels then continued to rise but did not achieve quite the same levels as normal. However, cyclin B protein levels did not rise after radiation despite apparently high levels of cyclin B mRNA until the G2 delay was over. Thus, there appears to be an additional regulatory mechanism affecting mainly cyclin B protein after irradiation in G2. Aberrations in p34cdc2 phosphorylation status at a time when p34cdc2 is complexed with cyclin B could also lead to G2 arrest and may be the point at which the gene rad9 exerts its control (54). Any of these alterations could potentially lead to the loss of p34cdc2 H1 kinase activity, which Lock and Ross (38) have demonstrated occurs after either ionizing radiation or treatment of cells with etoposide. O’Connor et al. (44) treated lymphoma cell lines with nitrogen mustard and found that the G2 delay induced in these cells was correlated with accumulation of phosphorylated p34cdc2 in the presence of cyclin B. Lock (58) has also reported similar results after treatment of CHO cells with etoposide, and Tsao et al. (59) found that camptothecin treatment of Hela cells also led to reduced dephosphorylation of p34cdc2. They also found cyclin B levels to be unaltered, although they noted synthesis of cyclin B to be reduced after camptothecin treatment. Thus, the supposition in these cases is that the dephosphorylation processes have been blocked as a consequence of the DNA damage leading to a G2 delay. The ultimate loss of p34cdc2 H1 kinase activity would be anticipated to lead to a division delay as is seen after DNA damage. Thus, the induction of G2 arrest after DNA damage appears likely to be a complex series of events at the molecular level involving alterations in gene expression at both the RNA and protein levels and alterations in enzyme activation via phosphorylation and dephosphorylation. The experiments reported here on the regulation of cyclin mRNA expression may contribute to our understanding of this complex phenomenon.

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