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Repair of chromosome and DNA breaks versus cell survival in Chinese hamster cells

J. BUSSINK†, P. J. TOFILON and W. A. BROCK*

Abstract. Clonogenic and non-clonogenic parameters of cell survival were compared in irradiated Chinese hamster cells. Clonogenic survival, chromatid break and repair kinetics, as well as DNA damage and repair, were assessed in synchronized cells in different parts of the cell cycle. G_2 chromatid damage and repair was examined in metaphase chromosomes of cells irradiated during S and G_2 phase, treated with or without inhibitors of DNA repair. Bromodeoxyuridine labelling of S phase cells starting at the time of irradiation made it possible to determine precisely, while scoring metaphase chromosomes, whether cells were irradiated in mid S, late S, or G_2 phases of the cycle. The results showed that chromatid breaks induced in S phase are efficiently repaired until the moment cells progress into G_2, when repair stops abruptly. Chromatid damage in G_2 phase is not repaired. On the other hand, DNA double-strand breaks are repaired in all phases of the cycle, even during G_2 phase which has no concurrent chromatid break repair. Finally, there is no consistent correlation between chromatid damage and repair, DNA damage and repair, and cell survival, thus indicating that the interaction of different parameters of radiosensitivity must be better understood for them to be useful predictors of cell survival.

1. Introduction

Assessing the radiosensitivity of cells by clonogenic survival assay provides a direct measure of the fraction of cells capable of forming macrocolonies after a given dose of radiation. Clonogenic assays, therefore, have the advantage of being both relevant and relatively free from artifacts. However, clonogenic assays are not feasible if cells fail to proliferate in culture as distinct colonies, if they have very low cloning efficiencies, or, as is the case in primary and early passage cultures, the cell population is heterogeneous. Clonogenic assays also fail to identify specific

mechanisms that account for radiosensitivity differences between different cell populations. As a result, the development of alternative, mechanism-based assays of radiosensitivity has been proposed and tested. Such assays are usually based upon known mechanisms of radiation cell killing, including radiation induced chromosome aberrations (Dewey *et al.* 1971, Carrano 1973), micronuclei formation (Midander and Revesz 1980), DNA damage and repair (Kelland *et al.* 1988, Schwartz *et al.* 1988, Blocher *et al.* 1991, Cassoni *et al.* 1992, Giaccia *et al.* 1992, Smeets *et al.* 1993), apoptosis (Fisher 1994), cell cycle phenomena (Su and Little 1993, McIlwrath *et al.* 1994), or repair mechanisms (Elkind 1985, Ward 1986, Kelland *et al.* 1987, Iliakis *et al.* 1988, Schwartz *et al.* 1988). The accuracy of non-clonogenic assays, relative to clonogenic ones, depends upon the cell types and mechanisms assessed. Unless those specific features that happen to distinguish the radiosensitivity differences of the cells being compared are measured, there will be no correlation with clonogenic survival. Thus, a chromosome break assay would be expected to reveal radiosensitivity differences between two normal diploid fibroblast strains, since normal fibroblasts typically die by reproductive death. On the other hand, chromosome break assays would *not* accurately detect radiosensitivity differences between cell lines that have variable levels of radiation-induced apoptosis, because the apoptotic mechanism would destroy chromosomes in lethally irradiated cells before they could be scored. Nevertheless, apoptosis is not typical of many established cell lines (Radford 1991) and the induction and repair of chromosome damage has become a very useful and accurate method to determine radiosensitivity (Joshi *et al.* 1982). And, since chromosome lesions depend upon the induction of DNA strand breaks, assays of DNA damage and repair have also been considered relevant. However, assays of DNA damage have generally lacked the necessary sensitivity to detect the one or more residual double-strand breaks

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that constitute a lethal lesion (Smeets *et al.* 1993). Therefore, superlethal doses of radiation are used and it is assumed that the relative number of breaks measured is proportional to what would occur after small doses. Attempts to find alternatives for measuring clonogenic survival, therefore, have not yet identified a generally useful approach, and ultimate success must take into account the mechanistic determinates of radiosensitivity and their interactions.

To explore further in detail the relationship between clonogenic and non-clonogenic parameters of radiation sensitivity, we compared measurements of cell survival, chromosome aberrations, and DNA damage and repair in Chinese hamster ovary cells (CHO AA8), with a particular emphasis on G_2 phase chromatid damage and repair. CHO cells were synchronized by aphidicolin block and centrifugal elutriation, and assayed for survival, chromosome aberrations, and the induction and repair of DNA double-strand breaks. The results show that while chromatid aberrations generally correlate with survival, the repair kinetics of those breaks can be misleading. The number of DNA double-strand breaks induced and their repair kinetics do not correlate with either survival or chromatid breaks. In fact, DNA strand break repair can be efficient, even in cells that do not repair chromatid breaks.

2. Materials and methods

2.1. *Cell culture*

Chinese hamster ovary cells (CHO-AA8) were grown in Hsu's modification of McCoy's 5A medium, supplemented with 15% foetal calf serum and 2 mM L-glutamine (Sigma Chemical Co., St Louis, MO, USA). Cultures for chromatid break determinations were seeded at a density of $8 \cdot 0 \times 10^5$ cells in 25-cm^2 flasks 2 days prior to the experiment, in order to assure exponentially growing populations.

2.2. *Irradiation for chromatid break and cell survival determinations*

Cultures were irradiated with a ^{137}Cs source at a dose-rate of 2·08 Gy/min. For the chromatid break experiments, cells were irradiated at 37°C. For survival assays, synchronized cells were irradiated on ice.

2.3. *Repair inhibition*

Chromatid break repair was inhibited by the addition of 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) to a final concentration of 200 μM 30 min prior to irradiation (Jayanth and Hittelman 1991).

2.4. *S phase labelling*

S phase cells were labelled with 1·0 μM bromodeoxyuridine (BrdUrd; Sigma), added immediately after irradiation (Terry *et al.* 1991). The BrdUrd labelling was continuous until mitotic cells were collected.

2.5. *Preparation of metaphases*

Mitotic cells were collected at different times after irradiation (90, 120, 150, 180 and 210 min) by incubating them for 1 h with 0·05 μg/ml colcemid (GIBCO, Long Island, NY, USA), followed by vigorous shaking. As a control, the cells remaining after shake-off were collected by trypsinization and found to contain the same level of chromatid damage as the cells selected by shake-off alone. After shake-off, the cells were centrifuged (5 min at 200 *g*), resuspended in 1% sodium citrate for 10 min at room temperature, and centrifuged again. The wet pellet was next fixed by gentle resuspension in 3 ml Carnoy's fixative (methanol: acetic acid; 3:1). Fixation was repeated with three additional Carnoy's washes before the fixed cell suspension was dropped onto wet microscope slides. The slides were allowed to air dry overnight before staining.

2.6. *Immunocytochemical staining*

Cells were firmly attached to the microscope slides by heating in a dry oven for 30 min at 110°C. The DNA was partially denatured in 4 N HCl for 20 min, followed by two rinses in 0·1 M sodium tetraborate and a 5-min rinse with phosphate-buffered saline (PBS). The slides were flooded with a 1:2000 dilution of IU-4 (mouse anti-IdUrd/BrdUrd; Caltag Laboratories, South San Francisco, CA, USA) in PBS with 1% bovine serum albumin and 0·5% Tween-20, and then incubated for 60 min at 37°C. After three 5-min rinses in PBS the slides were incubated with biotinylated anti-mouse IgG for 30 min at 37°C.

followed by three PBS rinses. Next, the slides were incubated with the avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature, followed by three PBS rinses. They were next treated with 1% diaminobenzidine tetrahydrochloride and 0·03% H_2O_2 in PBS. Finally, the slides were couterstained for 10 min with filtered Giemsa at pH 10.

2.7. *Cell synchrony*

Synchronized CHO cell cultures were prepared by different combinations of aphidicolin block of cells released from confluent cultures, cell cycle progression after release from aphidicolin, centrifugal elutriation, and mitotic shake-off. The details of the techniques for the various cell cycle phases are described elsewhere (Meistrich 1977, Meistrich *et al.* 1977, Bussink *et al.* 1995). The cell cycle distribution of each population was determined by multiple-parameter flow cytometry, using DNA content and BrdUrd incorporation (Terry *et al.* 1991).

2.8. *Clonogenic survival*

Cell survival was determined by clonogenic assay. Colonies were stained with crystal violet after 8 days in culture and the dishes were coded and scored blindly. Data were computer fit using a linear-quadratic model.

2.9. *DNA damage*

DNA double strand break repair kinetics was measured by pulsed-field gel electrophoresis (PFGE) according to a previously published protocol (Bill *et al.* 1992). Briefly, the cells are irradiated in suspension to a total dose of 40 Gy using a ^{137}Cs source with a dose-rate of 40 Gy/min. After irradiation, the cells were incubated in spinner flasks at 37°C. At different times after irradiation, aliquots of cells were removed from the incubator, washed with PBS at 4°C, and 0·5% agarose plugs, containing 2×10^5 cells/ml, were prepared by mixing equal volumes of the cell suspension and 1% low melting-point agarose (InCert; FMC Corp., Rockland, ME, USA). The plugs were allowed to gel at 4°C and then placed into lysis buffer (500 mM EDTA, 2% Sarkosyl, 1 mg/ml proteinase-K, pH 9·0) at 50°C for 16 h, followed by

five 90-min washes in PBS. The plugs were placed into a 0·5% agarose gel in 0·5 × TBE (45 mM Tris, 45 mM boric acid, 2 mM EDTA, pH 8·3; TBE). Contour clamped homogeneous electric field gel electrophoresis was performed using a continually circulating running buffer of 0·5 × TBE at 25°C. The gel was run at 40 V for 18 h, using a switching interval of 75 min. The hexagonal array of electrodes allows for the current to be alternated at 120°. After electrophoresis, the gel was stained with ethidium bromide (0·1 µg/ml) and the total DNA remaining in each plug versus DNA that migrated out and was determined by scintillation counting. The percentage of total DNA migrating from each plug relative to controls was taken as the percentage of DNA damage remaining.

2.10. *Chromatid breaks*

Chromatid breaks were scored according to the criteria of T.C. Hsu (personal communication). Briefly, chromatid damage is considered to be a break if the size of the gap is greater than the width of the chromatid or if there is misalignment or displacement of the chromatid axis across a gap. Slides were coded and scored blind.

3. Results

3.1. *Chromosome damage and repair in late S and G_2 phase cells*

Chromatid break repair kinetics was determined in metaphase chromosomes at various times after irradiation. Cultures were irradiated, incubated for at least 30 min to allow mitotic cells to pass through metaphase, and colcemid blocked for an additional 60 min. Therefore, at least 90 min elapsed between the time of irradiation and the first time point. Thus, the level of damage at 90 min was a function of the number of induced breaks and the degree of repair over a 90-min period. Therefore, to assess the number of initial breaks induced by 1·0 Gy at time zero, F-ara-A was added at a concentration known to inhibit chromosome break repair in these cells (Jayanth and Hittelman 1991). Figure 1 shows that 1·0 Gy resulted in 2·5 chromatid breaks per cell remaining 90 min after irradiation with little or no repair observed up to 120 min. From 120 to 210 min, the number of chromatid breaks decreased linearly.
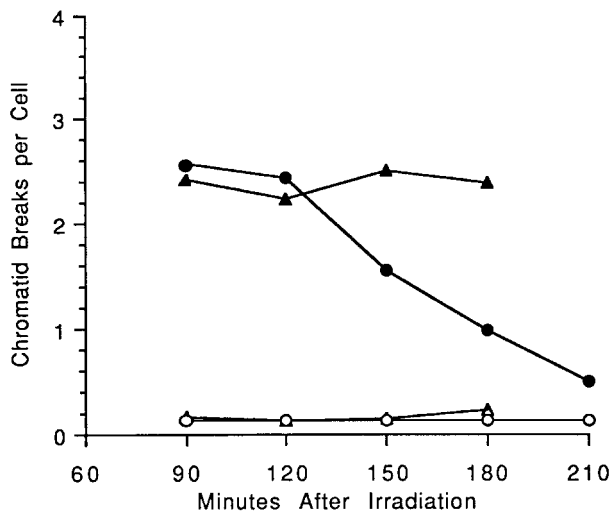
Figure 1.   Chromatid break frequencies in metaphase cells at various times after 1·0 Gy, with or without repair inhibition by 200 μM F-ara-A, added 30 min prior to irradiation. ○, Untreated cells; △, F-ara-A only; ●, 1·0 Gy only; ▲, F-ara-A + 1·0 Gy.

returning to near control levels by 210 min. F-ara-A-treated cultures showed the same number of chromatid breaks at 90 min, but the level stayed constant through 180 min, indicating that chromosome break repair had been blocked. Assuming that repair was inhibited from time zero, extrapolation of the F-ara-A curve to the ordinate can be taken as an estimate of the initial chromatid breaks induced by 1·0 Gy, approximately 2·5 breaks/metaphase. If this is true, the irradiated cells without repair inhibition did not repair chromatid breaks until 2 h after irradiation. Initially, this was viewed as a potential example of an induced or adaptive repair mechanism, but it was also possible that G_2 phase cells did not repair chromatid breaks and that the linear decline in break frequency after 2 h was due to the progression of repair competent S phase cells to metaphase.

3.2. *Progression kinetics of S and G_2 phase cells to metaphase*

To determine the cell cycle position of individual metaphase cells at the time they were treated, BrdUrd was used to label S phase cells. The presence or absence of BrdUrd staining in metaphase chromosomes thus distinguished between metaphases treated in S versus G_2 phase of the cycle. In fact, by estimating the fraction of chromosome surface area positive for BrdUrd, metaphases were identified as having been treated

in early S, mid S, or late S phase. The histogram shown in Figure 2a is the result of this approach with unirradiated cells. The histogram shows the distribution of metaphases in which BrdUrd was added in G_2 (0% labelled), S/G_2 border (minimal label), mid-to-late S (5–50%), and early-to-mid S (50–100%). Ninety min after BrdUrd addition, 85% of the metaphases were unlabelled, indicating that they were in G_2 at the time of BrdUrd addition; the remaining cells were in late S. The later time points show a progressive decrease in G_2 cells with corresponding increases in earlier and earlier S phase, that is, increasing BrdUrd staining.
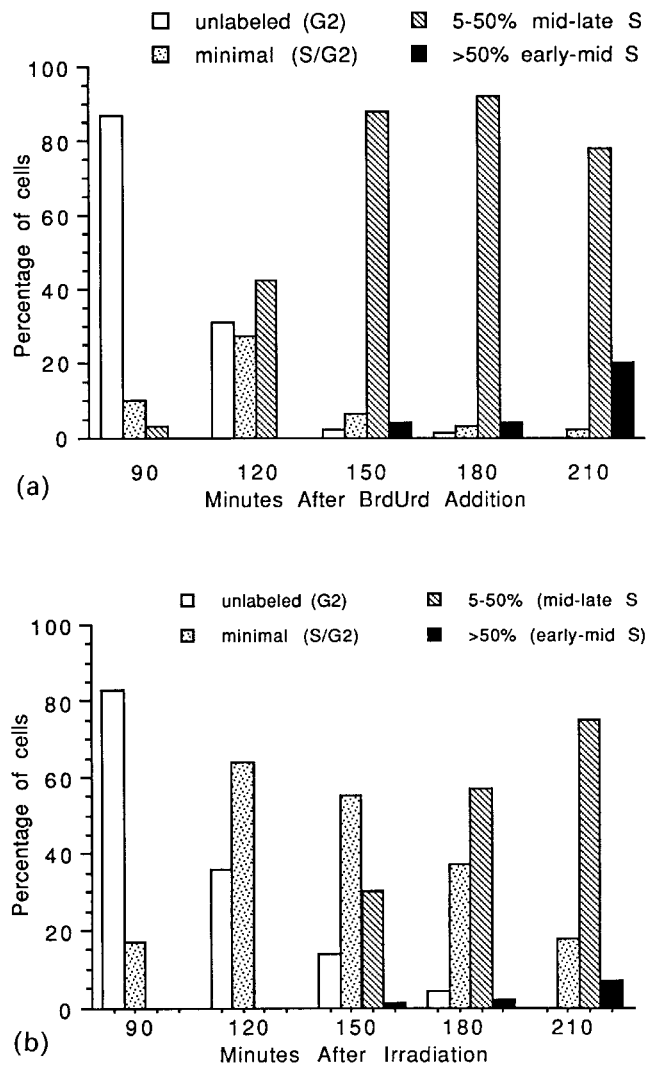

Figure 2.   Degree of BrdUrd staining in metaphase chromosomes at various times after the addition of 1·0 μM BrdUrd. The degree of BrdUrd staining indicates the position of the cell cycle at the time of BrdUrd addition. (a) Unirradiated cultures; (b) cultures irradiated with 1·0 Gy followed by immediate addition of BrdUrd.

Figure 2b shows the result from cultures irradiated immediately prior to BrdUrd addition. BrdUrd label was added after irradiation in order to avoid its sensitizing effects. As expected, the progression of cells from G_2 and S phase was somewhat slower, due to radiation induced cell cycle delays. In contrast to unirradiated cultures, the only S phase cells that had progressed to metaphase, 90 to 120 min after irradiation, were labelled minimally, indicating that they were in the very final minutes or seconds of S phase at the time of treatment. After 120 min a similar progression of increasing numbers of S phase cells appeared in metaphase. This labelling method thus provides a means to determine, on an individual cell basis, the cell cycle status of each metaphase at the time it was irradiated.

3.3. *Chromatid break repair kinetics in S and G_2 phase cells*

Using the approach just described, cultures were treated with 1·0 Gy followed by immediate BrdUrd addition. The cells were allowed to progress for various times and each metaphase was assessed for both BrdUrd incorporation and the number of chromatid breaks remaining. The results are shown in Figure 3. The top curve shows that the
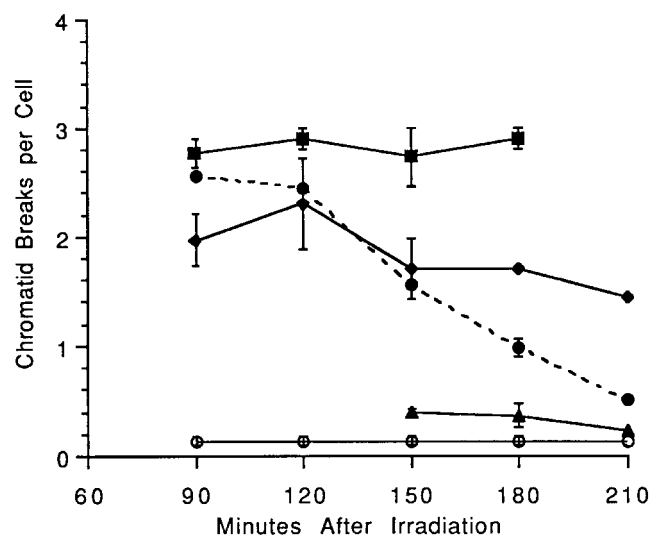

Figure 3. Chromatid break frequencies in metaphase cells at various times after 1·0 Gy, scored as a function of the degree of BrdUrd labeling to indicate the cell cycle phase of each metaphase at the time of treatment. ○, Untreated controls; ■, G_2 phase cells, BrdUrd negative; ◆, S-G_2 boundary cells, minimal BrdUrd staining; ▲, S phase cells, BrdUrd positive; ●, all cells combined at each time point. The numerical data for this figure are given in Table 1.

number of chromatid breaks induced in G_2 cells does not change between 90 and 180 min after irradiation, clearly indicating that G_2 cells do not repair radiation induced chromatid breaks. There are no data for G_2 cells at 210 min because all G_2 cells would have passed through metaphase before the addition of colcemid. On the other hand, cells with positive BrdUrd labelling show almost complete repair of chromosome damage by 150 min. It is not certain if the repair occurred only in S phase, before these cells entered G_2, or if, in contrast to irradiated G_2 cells, they were able to continue repairing breaks after entering G_2. However, the metaphases with minimal BrdUrd labelling suggest that repair capability ends abruptly as cells enter G_2. These cells were at the S/G_2 border at the time of irradiation, having only minutes or seconds remaining before entry into G_2. The number of breaks at 90 min (approximately two breaks/cell) is reduced relative to the G_2 cells, but the level of these breaks remains relatively constant up to 210 min. It thus appears that these cells repaired some chromatid breaks during the last moments of S phase but, since there is essentially no change in the number of breaks over the remaining time, the repair process must have disappeared as these cells entered G_2. These experiments clearly show an efficient repair mechanism throughout S phase that does not exist in G_2.

The data plotted in Figure 3 are given in Table 1, which lists the number of chromatid breaks and the number of metaphases scored at each time point. Because of progression through the cell cycle, the number of metaphases representing different parts of S and G_2 will be variable over time. For example, cells in mid-S at the time of treatment did not reach metaphase before 120 min, while G_2 cells were decreasing in numbers after 90 min, due to the short duration of G_2. Therefore, it was not possible to score 50–100 metaphases for each data point, which is usually considered to be ideal.

To determine if the lack of a repair capability is related to pre- versus post-replicated chromosome regions, chromatid breaks in irradiated, BrdUrd labeled, and late S phase cells, were classified as being within BrdUrd positive versus negative regions. It was assumed that the majority of BrdUrd negative areas contain post-replication DNA. By estimating the fraction of chromosome areas positive for BrdUrd and the number of breaks in each region, it was determined that the probability of a break occurring in replicated versus unreplicated areas is approximately equal

Table 1.   Degree of BrdUrd labelling and the number of chromatid breaks per cell in metaphase chromosomes at various times after treatment with 1·0 Gy

| BrdUrd labelling* | Chromatid breaks/cell ± SE (min after irradiation) | | | | |
	90	120	150	180	210
G$_2$	2·77 ± 0·13	2·90 ± 0·10	2·73 ± 0·27	2·90 ± 0·10	
0%	$n = 66$	$n = 34$	$n = 25$	$n = 5$	$n = 0$
S/G$_2$	1·97 ± 0·17	2·30 ± 0·30	1·70 ± 0·20	1·70 ± 0·10	1·44 ± 0·10
<5%	$n = 12$	$n = 44$	$n = 60$	$n = 47$	$n = 18$
early-late S			0·39 ± 0·10	0·36 ± 0·11	0·23 ± 0·10
5–100%	$n = 0$	$n = 0$	$n = 51$	$n = 127$	$n = 83$
All cells	2·56 ± 0·10	2·44 ± 0·10	1·55 ± 0·10	0·98 ± 0·10	0·50 ± 0·10
	$n = 78$	$n = 78$	$n = 136$	$n = 179$	$n = 101$

*The degree of labelling is an estimate of the percentage of total metaphase chromosome surface area stained positive for BrdUrd. See text for additional details.

(data not shown). If the induction of breaks is random with respect to pre- and post-replication areas, then the repair process must be the same throughout the chromatin.

3.4. *DNA double-strand breaks and repair*

In order to measure DNA damage and repair throughout the cell cycle, cultures were labeled with ^{14}C-thymidine before they reached confluency and were then synchronized by different combinations of release to low density, aphidicolin block at G$_1$/S, cell cycle progression, and centrifugal elutriation, as described. Briefly, mitotic cells were collected by shake-off techniques, G$_1$ and G$_2$ cells were purified by elutriation of cultures incubated for 8 h after release from an aphidicolin block, and S phase

Table 2.   Average cell cycle distributions of the synchronized cell populations used for DNA damage and repair, and survival assays

| Cycle phase | Cell cycle distribution (%)* | | | |
	G$_1$	S	G$_2$	M
G1	81	15	4	0
(range)	(69–90)	(8–31)		
S	2	62	36	0
	(1–3)	(49–80)	(17–45)	
G$_2$	1	28	67	4
		(27–33)	(64–73)	(2–6)
Mitotic		10†		51‡
		(9–10)		(48–53)

*Determined by multi-parameter flow cytometry as described in the Materials and methods, except for the mitotic cell fraction.
†Determined from BrdUrd labelling index on cytology preparations.
‡Determined from mitotic index on cytology preparations.

cells were obtained by incubating cultures for 4 h after aphidicolin release. The cell cycle distribution of each fraction was determined by dual parameter flow cytometry, measuring both DNA and BrdUrd incorporation; BrdUrd labelling is essential for accurately determining the position of cells that are at the beginning and end of S phase (Dutrillaux *et al.* 1991, Bussink *et al.* 1995). Table 2 shows the cell cycle distributions of separate preparations of G$_1$, S, G$_2$, and M phase cells used for DNA damage analysis and cell survival measurements. Each purified population was irradiated with a total dose of 40 Gy and the repair of DNA double-strand breaks (dsb) was determined by pulsed-field gel electrophoresis. Table 3 shows that the amount of initial damage was similar in G$_1$, G$_2$, and M phase enriched cultures. S phase cells appeared to have sustained less initial damage, although the differences are not significant due to the degree of variation in repeated measurements with S phase cultures. Figure 4 shows that repair kinetics in G$_1$, G$_2$, and S phase cells is the same. Even though the cells were somewhat heterogeneous in their cell cycle distribution (Table 2), the various fractions were enriched enough to conclude that G$_2$ cells repair DNA double-strand breaks. The purified

Table 3.   Initial DNA damage induced by 40 Gy as determined by the percentage of total DNA migrating from the plug during electrophoresis

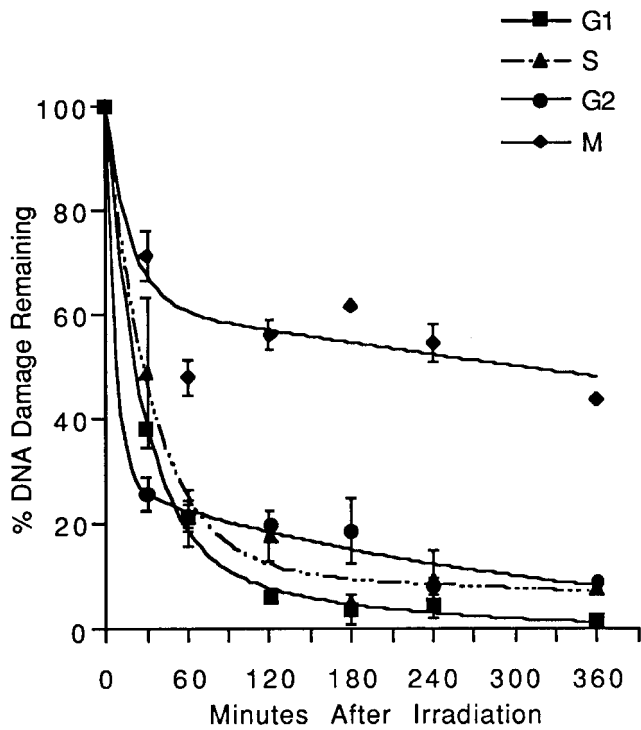| Initial damage | Purified fraction | | | |
	G$_1$	S	G$_2$	M
Mean (%)	50·0	37·8	52·3	55·4
Range	45·0–56·1	30·0–42·5	47·3–60·0	54·7–56·0
SEM	3·3	3·9	3·9	0·65
CV (%)	11·3	17·9	13·0	1·7

Figure 4. DNA double-strand breaks and repair in synchronized CHO cells in various phases of the cell cycle, determined by pulsed-field gel electrophoresis.

Figure 5. Clonogenic survival of irradiated synchronized CHO cells in various phases of the cell cycle. △, S phase; ○, G_2 phase; □, G_1 phase; ◇, M phase.

G_2 fraction contained an average of 67% G_2 cells (Table 2) and it shows the same rate of repair as S phase and G_1 phase cells. We, therefore, conclude that G_2 phase cells must account for at least part of the DNA repair in that fraction. Whether or not G_2 cells repair DNA damage at the same or a slower rate than cells in other phases of the cycle cannot be determined from these experiments. Only mitotic cells appeared different from the other cell cycle phases, since only 40–50% of their initial damage was repaired, likely related to their state of chromosome condensation.

3.5. *Clonogenic survival measurements*

Cell survival curves were generated for the same populations used for DNA damage and repair measurements and the results are shown in Figure 5. As expected, S phase was the most radioresistant, while G_1 and G_2 phase cells were more sensitive and not significantly different from each other. Again, the purity of the G_2 fraction ranged from 60 to 70%, compared with 80–90% for G_1, but the purity is adequate to demonstrate that G_1 and G_2 cells are not drastically different in radio-
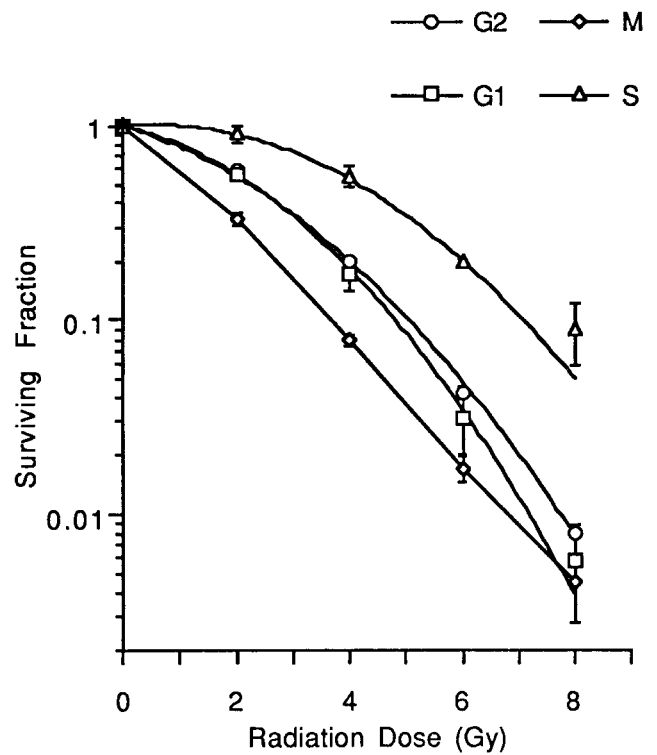
sensitivity. Mitotic cells were the most sensitive with log-linear survival curves.

4. Discussion

The results of this study show that correlations between different parameters of radiosensitivity in Chinese hamster cells is dependent upon the phase of the cell cycle in which they were measured. The relative degrees of chromosome and DNA break induction and repair, as well as clonogenic cell survival, change throughout the cell cycle, thus emphasizing that non-clonogenic endpoints of radiation sensitivity must be used with caution. The most dramatic differences were observed in S and G_2 phase cells. While chromosome breaks are efficiently repaired in S, there is no repair of them in G_2, even though DNA breaks are repaired in both phases of the cell cycle. Since it is commonly assumed that both DNA strand breaks and chromosome breaks are related mechanisms and that both contribute to cell lethality, the differences in these parameters were carefully studied in the S and G_2 phases.

The data in Figure 1 suggest that the repair of chromosome damage in G_2 is very slow or delayed for the first 2 h after irradiation and then rapid repair begins. This raised the possibility of a radiation induced repair mechanism, which has been suggested by others (Shadley and Wolff 1987, Wolff *et al.* 1988, Joiner *et al.* 1993). This observation led to a more complete analysis of chromosome break repair in S and G_2 phase cells. However, by using BrdUrd to differentiate between mitoses irradiated in S versus G_2 (Figure 2), it was determined that the apparent rapid repair after 2 h was due to the mitotic accumulation of cells that had been treated in S phase. The results, therefore, unequivocally demonstrate that chromatid breaks are not repaired during any part of G_2 and that there is no evidence for a radiation induced repair mechanism. In addition, the BrdUrd staining technique allowed us to carefully examine changes in chromatid break repair as cells progress through the S/G_2 border. Metaphases with minimal BrdUrd staining had been irradiated at the very end of S phase and they had fewer breaks remaining at the initial time point (90 min) than did G_2 cells (no BrdUrd labelling), but the flatness of that curve indicates that no additional repair took place as those cells traversed G_2. It, therefore, appears that some repair occurred during the final few minutes or seconds of S phase, but the moment S phase was completed, repair ended. The cessation of repair is so abrupt that it suggests the possibility of some type of control mechanism. However, there are no additional data to support this notion.

A lack of repair in G_2 has not been previously reported for this cell line, possibly because the typically short duration of G_2 makes it difficult to differentiate between repair that takes place in S versus G_2 phase unless S phase cells are labelled. Hittelman and Pollard (1982) used the technique of premature chromosome condensation (PCC) to examine the repair of radiation induced breaks and gaps in this same cell strain (AA8). They reported that the number of PCC breaks and gaps remains constant for 30 min following irradiation, and that is followed by rapid repair. Those results are consistent with our findings except for the timing (30 versus 90 min), but that can be explained by technical differences. Unlike metaphase analysis of chromosome damage, the PCC method is not dependent upon cell cycle progression for visualization of damage. A G_2-PCC preparation is, therefore, composed of cells from all parts of G_2, early to late, while a metaphase preparation is a sequential analysis of chromosomes that become visible as the cells progress to metaphase after treatment. Thus, at early times after irradiation, metaphases represent cells that were treated in late G_2, while cells treated in S phase do not reach metaphase for at least 90 min. On the other hand, G_2 PCC spreads will contain increasing numbers of cells irradiated in S phase shortly after treatment and become significant within 30 min. This appears to account for the kinetic differences in the results, but both observations are consistent with no G_2 repair.

One consequence of no repair in G_2 would be an expectation of a simple exponential survival curve for purified G_2 cells, similar to that displayed by mitotic cells. Instead, a shouldered survival curve, almost identical to the survival of G_1 cells, was observed. This questions the validity of interpreting shouldered curves as always being due to the repair of radiation injury. However, it is possible that contamination of the purified G_2 cells with S phase cells could be responsible for the shape of the initial slope of the G_2 survival curve. Another potential consequence is related to the phenomenon of radiation induced cell cycle arrest, which can be inferred in these experiments from the delay in the appearance of irradiated BrdUrd labeled cells at metaphase relative to controls. G_2 arrest is thought to provide time for repair of DNA and chromosome damage before a cell enters mitosis (Darroudi and Natarajan 1987, Utsumi and Elkind 1991) and would be expected to increase cell survival. Support for this notion comes from reports that CHO cell killing is enhanced if the G_2 delay is abrogated by caffeine treatment (Darroudi and Natarajan 1987, Rowley 1992). How G_2 delay contributes to increased survival in cells that do not repair chromatid damage in G_2 is not clear.

A dissociation between DNA strand break repair and chromosome break repair was observed during G_2 phase. Although the G_2 populations in the DNA repair experiments were contaminated with some cells from other phases, it is clear that G_2 cells repair DNA breaks, because it is highly unlikely that the cells from other phases of the cycle could account for the high level of DNA repair observed in that fraction. It is also clear that the repair DNA breaks in G_2 does not translate into an ability to repair chromatid breaks. Using a similar cell strain (CHO 10B), Metzger and Iliakis (Metzger and Iliakis 1991) also found no relationship between radiation induced DNA double-strand break repair or PCC repair and cell survival. Similarly, Smeets *et al.* (1993) failed to find a correlation between cell survival and DNA

double-strand breaks in human tumour cells. On the other hand, other authors have found a correlation between the rate of DNA double-strand break repair and clonogenic survival (Schwartz *et al.* 1988, Giaccia *et al.* 1992). It is logical to assume that DNA strand break repair and chromatid break repair are important for cell survival following irradiation, but a more complete understanding of the mechanisms of those relationships and the exact parameters that should be measured is needed.

In conclusion, the relationship between radiation induced chromatid break repair and DNA break repair changes throughout the cell cycle but not always as would be predicted from changes in radiosensitivity. Although examples of significant correlations between combinations of these endpoints and cell survival have been found for different phases of the cell cycle and for different cell lines, their use is limited.

Acknowledgments

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