Abnormal Cytokinesis after X-Irradiation in Tumor Cells that Override the G_2 DNA Damage Checkpoint

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Abstract

X-irradiation–induced DNA damage perturbs the G_1, S, and G_2 phases of the cell cycle. The behavior of cells after they have experienced a DNA damage checkpoint delay is poorly characterized. We therefore examined the fates of irradiated tumor cells that have overcome a prolonged G_2 checkpoint delay. Most irradiated cells progressed through mitosis without significant delay, but failed to complete cytokinesis as they remained tethered to each other at the midbody. We observed that the movement of centrioles at the time of cytokinesis was impaired in the irradiated, bridged cells. We attribute the perturbation of centriole dynamics to the presence of chromatin bridges that spanned the daughter cells. The bridged cells exhibited different fates that included death, fusion that formed multinucleated cells, or another round of mitosis with no noticeable cell cycle delays. The presence of γH2AX foci in the bridge as well as in the separated nuclei indicated that cells were proliferating despite the presence of DNA damage. It seems that DNA damage checkpoints were not reactivated in cells that overrode a prolonged G_2 delay. Cells deficient in *ATM, H2AX, XRCC3*, or *ligase 4* exhibited a higher frequency of radiation-induced bridges than controls, suggesting that the DNA bridges resulted from inadequate DNA repair. These data show a previously unappreciated cytologic hallmark of DNA damage in dividing cells. Chromatin bridges that interfere with cytokinesis are likely to contribute to the replication failure and clonogenic death of cells exposed to irradiation. [Cancer Res 2008;68(10):3724–32]

Introduction

X-irradiation of cells produces double-stranded breaks or clustered DNA damage that are generally thought to be the lethal forms of DNA damage (1, 2). Lethal irradiation results in a variety of cellular responses. In some cases, DNA damage leads to the rapid induction of apoptosis resulting in cell death before adequate repair might have been possible. DNA damage leads to apoptosis in some cell types such as thymocytes, hematopoietic stem cells, endothelial cells, colonic crypt cells, and a subset of tumor cells often derived from radiosensitive tumors such as lymphomas or testicular carcinomas. In other cell types, DNA damage is more likely to result in permanent cell cycle arrest accompanied by markers for senescence, an effect that has sometimes been seen after the irradiation of tumor cell lines, but is often not the predominant outcome. In some cases, an event in which condensed chromosomes in mitosis disintegrate is termed a mitotic catastrophe, but this results less commonly after irradiation of tumor cell lines (3–6). The irradiation of many tumor cell lines results in clonogenic death not associated with rapid apoptosis, senescence, a permanent cell cycle arrest, or mitotic catastrophe (2, 5–9). Instead, this clonogenic death is characterized by abortive colony formation in which several cell cycles occur, culminating in either multinucleated cells, later cell death and apoptosis, or small colonies that do not continue to grow. The possibility of cell division after irradiation raises the question of whether the cells proliferate in the presence of DNA damage. Video microscopy has been used to record colony formation and cell death after irradiation, revealing that many types of tumor cells continued to divide for up to 10 generations with death of daughter cells occurring by apoptosis or necrosis (10–12). Syljuasen et al. have shown that these cells recognize DNA damage through DNA damage foci formation yet continue to divide (13). Here, we examined cell division in irradiated cells following a G_2 checkpoint delay. We can report that these dividing cells have a defect in cytokinesis that can be attributed to the presence of chromatin bridges that interfere with centriole dynamics that are normally required for abscission. The chromatin bridges, along with unrepaired DNA in the daughter nuclei, may contribute to the failure of these cells to continue to replicate.

Materials and Methods

Cell culture and irradiation. HeLa, T24, AT22IJE and AT22IJE-T, and immortalized MEFs (derived from p53−/− mice) either wild-type or ligase 4−/− (14) (kindly supplied by G. Iliakis, Institute of Medical Radiation Biology, University of Duisberg-Essen, Essen, Germany) were grown in a humidified 37°C incubator with 5% CO_2 level in DMEM supplemented with 10% fetal bovine serum, nonessential amino acids, and 2 mmol/L of L-glutamine. HeLa cells expressing the fusion proteins GFP-H2B or GFP-centrin as described (15, 16). AT22IJE (also named FTYZ5) and AT22IJE-T (also named FYPEB37) were kindly provided by Dr. R. Daniels (Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; ref. 17).

Cells were grown in 35 mm Glass Bottom Microwell Dishes (MatTek Cultureware) and incubated with 2 mmol/L of thymidine (Sigma) for 16 h and then thymidine was removed by washing thrice with medium (15, 18). Three hours after thymidine release, cells were irradiated with X-ray at the indicated doses and video recording was started 8 to 10 h later, or in some cases, 24 h later. Caffeine (Sigma) was added into the medium at the final concentration of 2 mmol/L, 1 h before video recording was started. Cells were maintained during recording in buffered medium at 37°C on a heated stage.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Small interfering RNA and transfection. All small interfering RNAs (siRNA, synthesized by Dharmacon; sequences can be provided on request) were transfected at the final concentration of 20 nmol/L using Hiperfect (Qiagen) according to the manufacturer's instructions. Typically, HeLa cells were grown on coverslips in six-well plates and transfected at 30% to 40% confluency. Coverslips were irradiated 48 h after transfection and then fixed 24 h postirradiation.

Antibodies. Rabbit polyclonal antibodies against Bub1, BubR1, Mad1, Cenp-F, and hSgo2 were raised by our own laboratory as described, and their specificities have been documented (16). Mouse monoclonal anti–phosphorylated histone H2AX (Ser^{139}) antibody was purchased from Upstate. Other antibodies used include monoclonal anti–α-tubulin (Sigma), chicken anti-hMgcRacGAP (R&D systems), mouse monoclonal anti-survivin (Novus biologicals), anti-Aurora B (BD Biosciences), anti-CRIK (citron kinase; BD Biosciences), and rabbit polyclonal anti-INCENP (Sigma).

Immunofluorescence microscopy. Cells were plated onto no. 1.5 glass coverslips, fixed for 7 min in freshly prepared 3.5% paraformaldehyde (in PBS; pH 7.0), extracted in "K" buffer [KB; 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, and 0.1% bovine serum albumin] plus 0.2% Triton X-100 for 5 min at room temperature, and rinsed in KB. Primary and secondary antibodies were diluted in KB and added to coverslips for 30 to 60 min at 37°C. All the secondary antibodies were conjugated to Alexa Fluor dyes (Molecular Probes). 4′,6-Diamidino-2-phenylindole (DAPI) was used to counterstain DNA at 5 ng/mL of final concentration.

Images were taken on an inverted microscope (Eclipse TE2000E; Nikon) equipped with a Cascade 512F camera (Roper). Videos of GFP/centrin HeLa cells were captured with a spinning disc confocal microscope (Ultraview; Perkin-Elmer) that consisted of a microscope (Eclipse TE2000S; Nikon) and a charge-coupled device camera (ORCA-ERG; Hamamatsu). Image processing was performed using ImagePro Plus 5.0 software as described by the manufacturer's guidebook. Metavue (Molecular Devices) software was used for processing and analysis of immunofluorescent images.

Results

Cells synchronized by a double thymidine block were released, irradiated in S phase, and their fates, after their exit from the G_2 DNA damage checkpoint, were examined by time-lapse videomicroscopy (~10 hours postirradiation). At least 80% of the unirradiated control HeLa cells completed cytokinesis (as defined as cell separation) within 100 to 400 minutes with a median time of 260 minutes. Control T24 cells had similar timing for cytokinesis with a broader range. The majority of irradiated cells completed mitosis after a prolonged G_2 delay and separated to form two daughter cells. The timing of mitosis from the onset of chromosome separation through formation of two nuclei was not significantly altered by irradiation (data not shown). However, the daughter cells remained connected by a cytoplasmic bridge. HeLa cells treated with 2 or 6 Gy remained joined for median times of no less than 790 and 920 minutes, respectively (Fig. 1*A* and *B*; Supplemental Movies 1 and 2). Irradiated T24 cells formed similar bridges (Fig. 1*Bc*).

The defect in abscission of the cleavage furrow was followed by a variety of different fates: cell death of one or both daughter cells, continued cell division by cells connected by a cytoplasmic bridge with the resultant cells also joined by a bridge, or cell fusion resulting in multinucleated cells (Fig. 1*C* and *D*; Supplemental Movie 2).

We determined that the cytoplasmic bridges of the irradiated cells contained DNA as revealed by DAPI staining and chromatin as revealed by H2B-GFP. As expected, DNA or chromatin was not detected in the cytoplasmic bridges of unirradiated post-anaphase cells (Fig. 2). Although dicentric chromosomes could occur as a result of radiation, they have not been reported to exceed 10% of the population (19), whereas in these experiments, up to 80% of the irradiated cells were connected by DNA bridges. We next compared the localization patterns of proteins associated with the midbody and cleavage furrow between control and irradiation-treated cells. No obvious differences were found after staining for Aurora B, INCENP, MgcRACGAP, CRIK, survivin, or MyosinIIB between the DNA-containing bridges of irradiated cells and the control cells (Fig. 3; Supplemental Fig. S1). The presence of DNA did not grossly affect the localization of proteins that are critical for cytokinesis and abscission.

Centriole dynamics play an important role in the completion of cell division. The mother centriole transiently moves to the midbody during cytokinesis and moves away prior to abscission (20, 21). We used spinning disk confocal microscopy to track the centriole dynamics in HeLa cells expressing GFP-centrin (Fig. 4; Supplemental Movies 3 and 4). In 19 out of 20 unirradiated cells, a centriole migrated to the midbody and then away prior to abscission. In 13 out of 16 irradiated cells, a centriole migrated to the midbody, but remained there. In two cells, we observed no centriole movement before the cells fused. In one cell, the centriole migrated as in unirradiated cells. We believe that the presence of chromatin in the bridges interfered with the proper migration of the centrioles, which is normally required for abscission.

The source of the chromatin bridges may be lagging chromosomes from a defective mitosis by the irradiated cells (16). To test whether lagging chromosomes themselves could result in persistent cytoplasmic bridges, we directly induced lagging chromosomes by depleting the kinetochore proteins, hSgo2 or CENP-F (16, 22). Both proteins have been shown to be essential for the proper attachment of chromosomes to the spindle and the resulting lagging chromosomes are due to the inability of the chromosomes to be properly segregated during anaphase. Although lagging chromosomes were produced in cells that were depleted of hSgo2 or CENP-F, neither treatment led to persistent bridges nor noticeable defects in cytokinesis (Fig. 5).

We next asked whether the abscission failure in irradiated cells was due to the activation of cell cycle checkpoints. Treatment with caffeine, which abrogates the G_2-M checkpoint, had no effect on the formation or persistence of these bridges (refs. 23–26; Fig. 5*E*; Supplemental Fig. S3*C*). Similarly, down-regulation of CENP-F, hSgo2, or BUBR1, which could bypass the mitotic checkpoint (27, 28), did not alter the formation or persistence of cytoplasmic bridges in irradiated cells (Fig. 5; data not shown). The localization patterns of the spindle checkpoint and kinetechore proteins BUB1, BUBR1, MAD1, hSgo2, and CENP-F during mitosis were indistinguishable between the irradiated and the control cells (Fig. 5; Supplemental Fig. S2). Thus, the source of the lagging chromosomes in irradiated cells does not seem to be derived from defective spindle and chromosome interactions during mitosis.

We next tested whether the chromatin bridges contained damaged DNA as determined by γH2AX foci formation. More than 90% of cells with bridges had detectable γH2AX foci after 0.5, 1.0, or 2.0 Gy of irradiation (Supplemental Fig. S4). In many cases, foci were clearly detected at the DNA bridge (Fig. 6*A*; Supplemental Fig. S3*A*). As γH2AX foci were also detected in both nuclei of the bridged cells, these observations indicate that the irradiated cells had initiated mitosis and progressed to the point of abscission despite the presence of recognized DNA damage. Time-lapse experiments showed pairs of daughter cells that were connected by a bridge to divide again and to form two more cells that remain connected to each other (Fig. 1*C* and *D*). The ability of the bridged

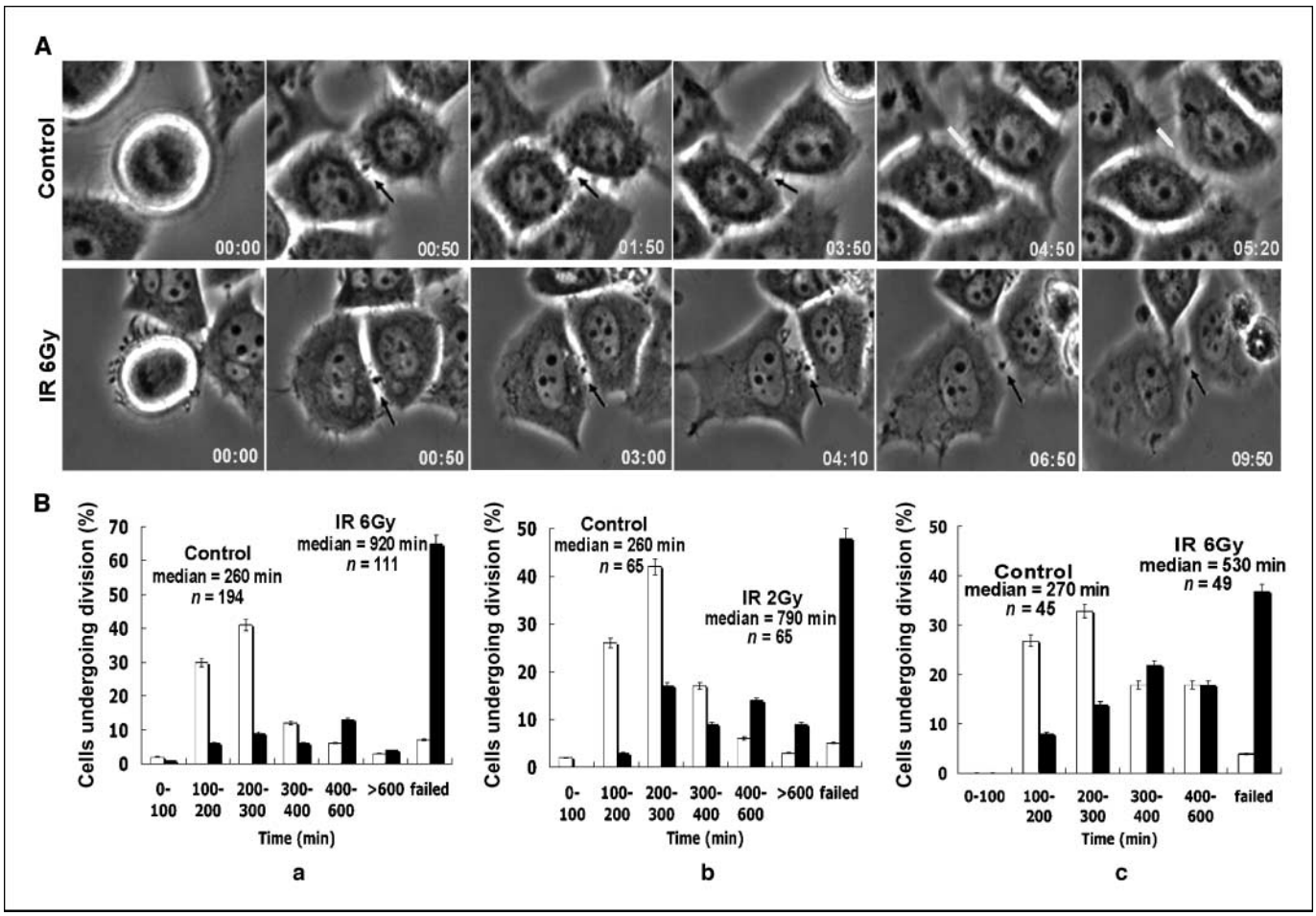

Figure 1. Ionizing radiation disrupts cytokinesis. *A,* images from time-lapse videos of mock (*Control*) and irradiated (*IR*) HeLa cells. Time 0 was defined as the first instance of separation of metaphase-aligned chromosomes. Control cells completed separation (*white arrow*) by 4 h and 50 min, whereas irradiated cells remain tethered at the midbody (*black arrows*). *B,* percentages of cells that completed cell separation at various times after onset of anaphase. Panels *a* and *b* compare separation times of control HeLa cells (*white columns*) to times observed after exposure to 6 and 2 Gy of irradiation (*black columns*); *c,* separation times for control and irradiated T24 cells.

cells containing DNA damage to continue to divide without noticeable cell cycle delays indicated that DNA damage checkpoints were not maintained despite persistent DNA damage recognized by foci (Fig. 6).

We next asked whether the formation of bridges might be due to unresolved damaged DNA that is normally modulated by DNA repair. Cell lines derived from a patient with ataxia telangiectasia, AT22IJE and AT22IJE-T, which contain a vector encoding *ATM* or empty vector, respectively, were irradiated and examined for bridge formation. After a dose of 6 Gy, the number of bridges observed in the AT22IJE-T (ATM−) cell line was >12-fold more than in the AT22IJE (ATM+) cell line. This suggests that the *ATM* DNA damage checkpoint pathway is involved in the formation of bridges (Fig. 6*B*). We next tested the role of H2AX, also an early component of the DNA damage response pathway, in bridge formation in irradiated cells. HeLa cells depleted of H2AX by siRNA transfection showed a 10-fold increase in the number of bridges and a >20-fold increase in the number of bridges after irradiation relative to unirradiated control siRNA-treated cells (Fig. 6*C*; Supplemental Fig. S3*B*). However, the frequency of bridges in the H2AX siRNA + irradiated cells were apparently lower than the control siRNA + irradiated samples. To address this apparent discrepancy in the response, we analyzed the fates of cells by time-lapse videomicroscopy. Interestingly, the time-lapse studies showed irradiated cells that were depleted of H2AX also died more rapidly in mitosis (Supplemental Fig. S3*C*), without progressing to cytokinesis. The reason for why cells depleted of H2AX die in mitosis is under investigation but this can explain why there were apparently fewer bridged cells after depletion of H2AX than control irradiated cells.

Next, we directly examined the roles of DNA repair pathways in bridge formation. We examined the contribution of homologous recombination by using cell lines deficient in *XRCC2* (*IRS-1*) and *XRCC3* (*IRS-1SF*). Wild-type and mutant cell lines were irradiated with 1 Gy and examined for the frequency of bridge formation. When compared with wild-type Chinese hamster ovary *aa8*, the *IRS-1SF* cell line showed a 3-fold increase in the number of bridges, whereas *IRS-1* showed a 2-fold increase. This suggests that bridge formation can be due to unrepaired DNA resulting from defects in homologous recombination. We then tested the role of the nonhomologous end-joining repair pathway in bridge formation. MEFs defective in DNA repair due to genetic elimination of *ligase 4* and immortalized by elimination of *p53* were examined for bridge formation. A lower dose of 1 Gy was used in these experiments as the mutant cells are highly radiosensitive. When compared with

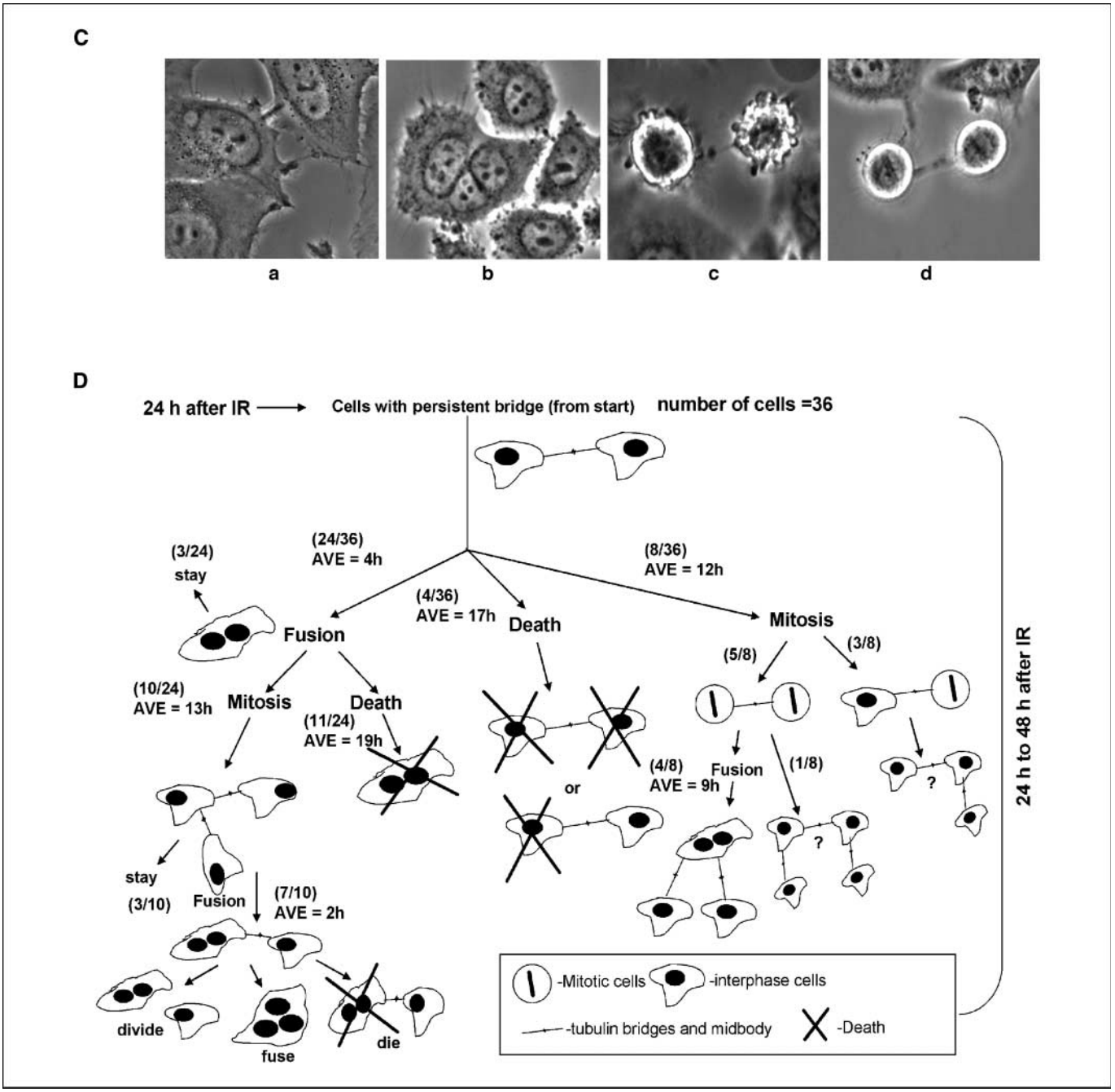

Figure 1 *Continued. C,* representative time-lapse images of four common fates for irradiated HeLa cells after a long delay (>900 min) of cytokinesis. *a,* persistent midbody; *b,* separated cells fused to form a multinucleated cell (*top right,* a pair joined by a persistent midbody); *c,* cells undergoing apoptosis while still connected by a bridge; *d,* two cells undergoing another round of mitosis while still connected. *D,* fate map for HeLa cells irradiated with 6 Gy.

wild-type immortalized MEF's, the *lig4*−/− MEF's showed a >3-fold increase in the number of bridged cells after irradiation (Fig. 6*D*). Thus, failure to repair damaged DNA by either homologous recombination or nonhomologous end-joining is likely responsible for the accumulation of chromatin bridges.

Discussion

DNA damage activates checkpoints that delay cell cycle progression to facilitate repair. In this study, we showed that cells irradiated with a lethal dose of irradiation eventually enter into mitosis with unrepaired DNA despite the G_2 checkpoint delay. We show that these tumor cells may undergo several rounds of mitosis despite the presence of DNA damage as determined by the presence of γH2AX foci. It seems that once irradiated cells overcome the initial G_2 checkpoint delay, they can continue to cycle in the face of DNA damage. These experiments confirm that these cells fail to block the cell cycle despite persistent DNA damage (13).

In some normal cells such as some fibroblasts or hematopoietic stem cells, DNA damage is followed by senescence that permanently blocks further cell cycle progression (6, 29, 30). In some

instances, apoptosis is activated by DNA damage thus obviating a need for cell cycle checkpoint. However, if apoptosis and DNA damage checkpoints are inhibited, these cells ultimately emerge with aneuploidy (8). Similar studies have shown that irradiated colon carcinoma cells have the same clonogenic survival in the presence or absence of caspase inhibitors (31). These data suggest that in transformed cells, inactivation of cell cycle checkpoints might be a survival mechanism that allows cell division in the face of persistent DNA damage. This is supported by the observation that the majority of the transformed cells examined in this study continued to proliferate for one or more cycles after exposure to irradiation.

Continued proliferation after DNA damage has been well documented by the labs of Dewey and others using video microscopy of irradiated cells. They noted that cells continued to divide often for many cycles with seemingly stochastic induction of apoptosis (11, 32). Chu et al. noted that apoptosis was enhanced by elimination of 14-3-3 or p21 (33). They did not comment on persistent bridges, but structures consistent with anaphase bridges can be seen in some of their publications. A more recent study estimated that cells with 10 to 20 double-strand breaks, which is equivalent to one or two chromosome breaks, will be released from a G_2 checkpoint delay and enter mitosis (34). We have directly examined irradiated cells that proceeded through mitosis and showed that the majority of these cells exhibit persistent chromatin bridges. Furthermore, we confirmed that these cells continue to divide for one to two cell cycles in the presence of damaged DNA. Thus, the loss of genome integrity through aberrant cell divisions is likely responsible for clonogenic failure as observed by Dewey et al.

Anaphase bridge formation has been noted in minority populations of unirradiated cells (35) but this did not seem to lead to cytokinesis failure. Inhibition of the abscission machinery will lead to persistent bridges (36). The mother centriole migrates to the midbody immediately before abscission during cytokinesis. Cells without centrosomes have cytokinesis abnormalities and failure (20, 21). Our observations of the dynamic behavior of GFP/centrin indicate that the normal movement of centrioles is disrupted in cells with chromatin bridges. This raises the possibility that the chromatin in the bridge acts as a physical barrier that interferes with centriole movement that is important for abscission and cell separation.

The different fates that are commonly seen in bridged cells following irradiation may be due to the random nature of the

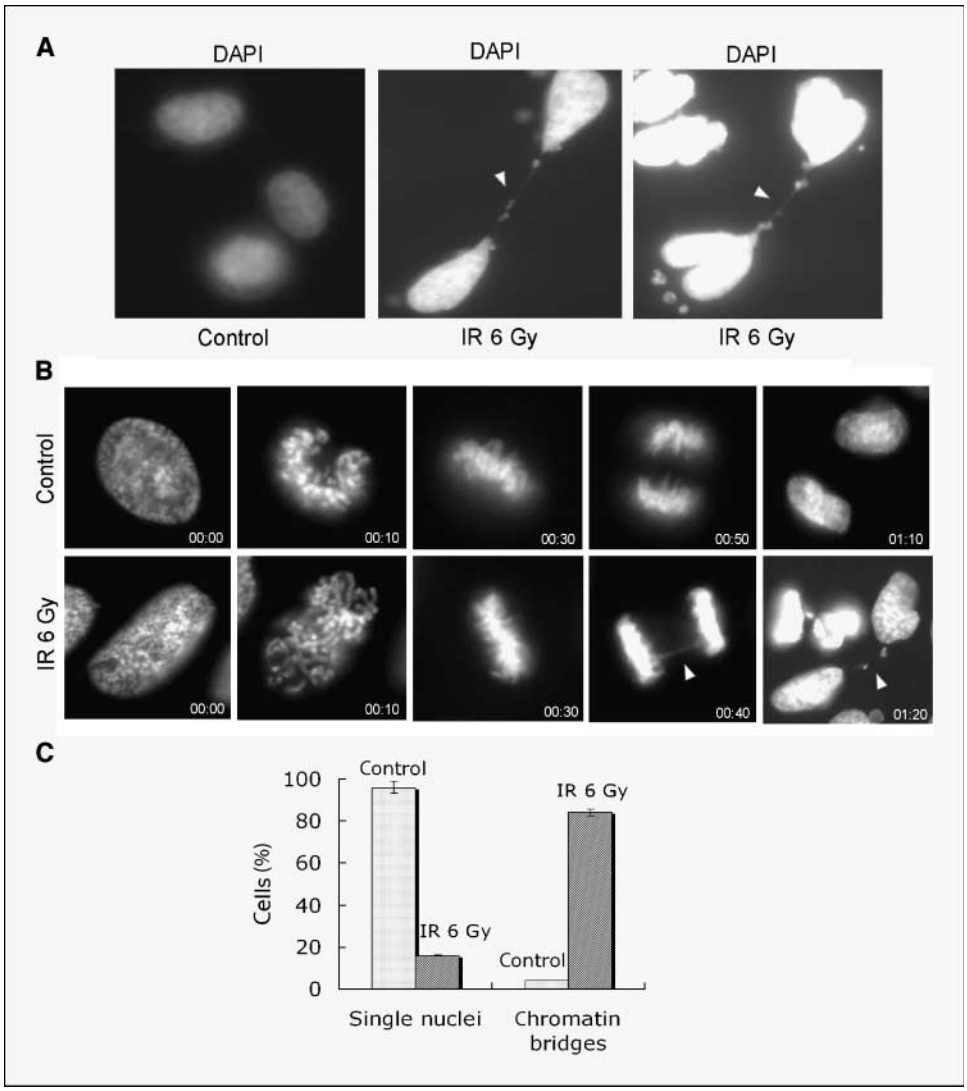

Figure 2. Bridges connecting irradiated cells contain DNA and chromatin. *A,* DAPI staining of control and irradiated HeLa cells. Bridges connecting the irradiated cells contain DNA. *Right,* an example in which exposure times were increased to reveal the DAPI-positive bridges. *B,* time-lapse of control-treated and irradiated HeLa H2B-GFP cells progressing through mitosis. H2B/GFP is detected in the bridges joining the irradiated cells. *C,* the number of HeLa cells that divided normally or remained connected by a chromatin bridge in control and irradiated samples were stained for DAPI, manually counted through the microscope, and plotted. More than 360 cells in total were counted from two independent experiments.

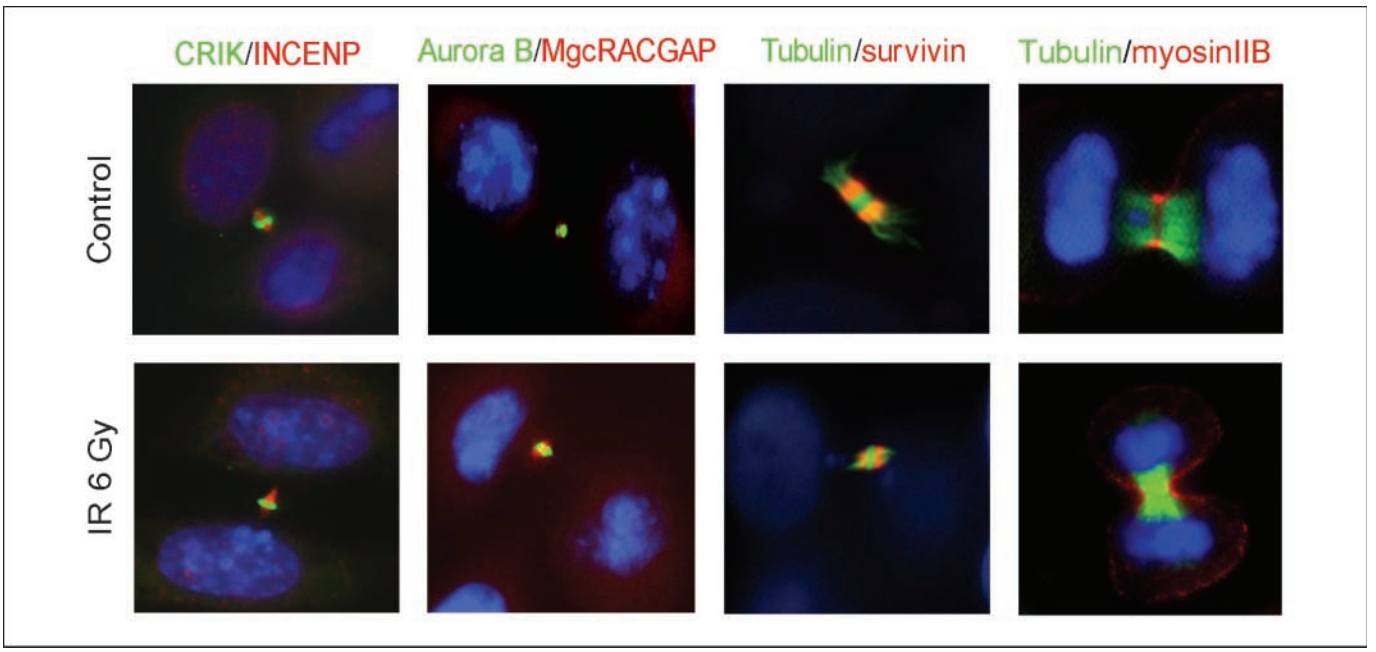

Figure 3. Localization of proteins known to be involved in cytokinesis are not affected by irradiation. Control or irradiated synchronized HeLa cells were costained with DAPI (*blue*) and immunostained for various chromosome passenger proteins, citron kinase, Aurora B, and MgcRACGAP (*pseudocolored*). Images of cells in mitosis were compared at similar times.

unrepaired DNA that is in the bridge as well as in the nuclei of the divided cells. A recent report by Norden et al. (37) showed that a NoCut pathway in the budding yeast, consisting of *Ipl1, Boi1*, and *Boi2* inhibits premature abscission to ensure that chromatin is cleared from the midzone before cytokinesis is completed. Inactivation of the NoCut pathway caused an increase in double-strand breaks that were believed to be due to premature cytokinesis. Thus, NoCut seems to be a new cell cycle checkpoint in yeast which ensures that chromosomes are completely segregated before cell separation. It remains to be seen if a NoCut

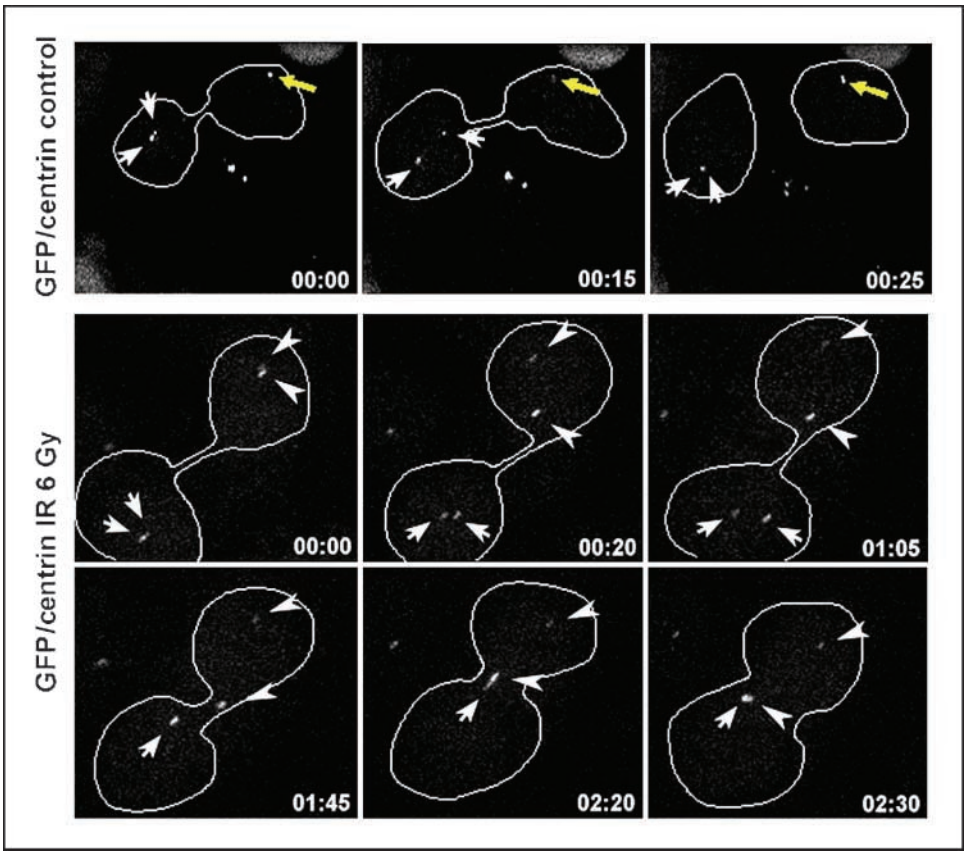

Figure 4. Effect of irradiation on centriole movement during cytokinesis. GFP/centrin was used to track the dynamics of centrioles during cytokinesis. HeLa cells expressing GFP/centrin were synchronized and the behavior of the GFP/centrin was monitored during mitotic exit by spinning disk confocal microscopy. The GFP/centrin signals (*arrows*) indicate the positions of each centriole in a pair. In the control, one centriole migrated towards the cleavage furrow (00:15) and then returned to re-form a pair (00:25). In this example, the returning centriole was positioned on top of the other centriole, thus appearing as a single spot. The other centriole pair (*yellow arrows*) did not separate. In the irradiated sample, one of the centrioles in a pair (*arrows* and *arrowheads*) migrated towards the cleavage furrow but failed to re-form with the original partner. In this example, a centriole from each daughter cell migrated to the midbody and remained there. These two daughter cells eventually fused.

system is conserved in mammalian cells. If such a system did exist, it does not seem to be linked to the conventional cell cycle regulatory pathways. Our time-lapse studies clearly showed that a significant portion of the bridged cells continued through another cell cycle.

There are several explanations for the presence of DNA in these bridges. Chromosomes without proper spindle microtubule attachments might be left behind in the center of a dividing cell and eventually become trapped in the cleavage furrow. However, direct disruption of kinetochore functions that produce lagging chromosomes in anaphase did not lead to detectable chromatin bridges. Thus, the chromatin bridges observed in the irradiated cells do not seem to arise from a defective mitosis. The increased frequency of the DNA bridges in *lig4−/−* MEFs that are deficient in nonhomologous end-joining, and *IRS-1SF* that is efficient in homologous recombination, suggests that the majority of bridges

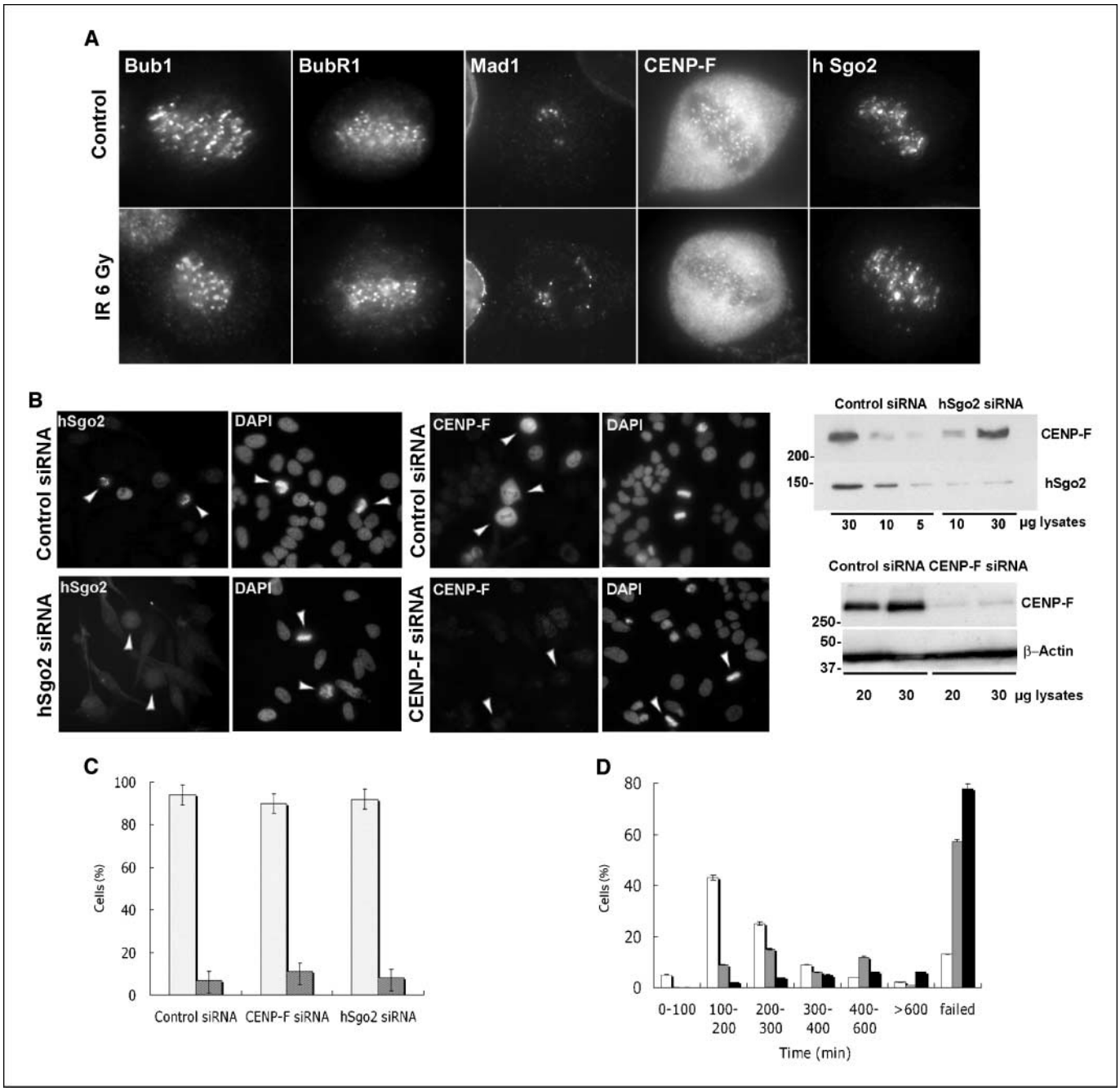

Figure 5. Evaluation of mitotic checkpoint proteins as potential targets for irradiation-induced chromatin bridges. *A,* synchronized HeLa cells mock-irradiated (*Control*) or treated with 6 Gy were stained after 24 h for the mitotic checkpoint proteins Bub1, BubR1, Mad1, CENP-F, and hSgo2. *B,* siRNAs were used to deplete cells of hSgo2 or CENP-F. Western blotting and immunofluorescence were used to confirm depletion of the targeted proteins in cell lysates as well as in mitotic cells (*arrowheads*) that were identified by DAPI staining. *C,* the number of bridged cells that were transfected with control, hSgo2, or CENP-F siRNAs was manually quantitated from four independent experiments. More than 300 cells in total were counted for each experiment. *D,* HeLa cells were treated with 2 mmol/L of caffeine and then mock-irradiated (*open column*) or irradiated with 2 Gy (*gray column*) or 6 Gy (*black column*) of X-rays. The time for completion of cytokinesis was measured from time-lapse video microscopy of the cells. A total of 250 cells from four movies were manually counted.

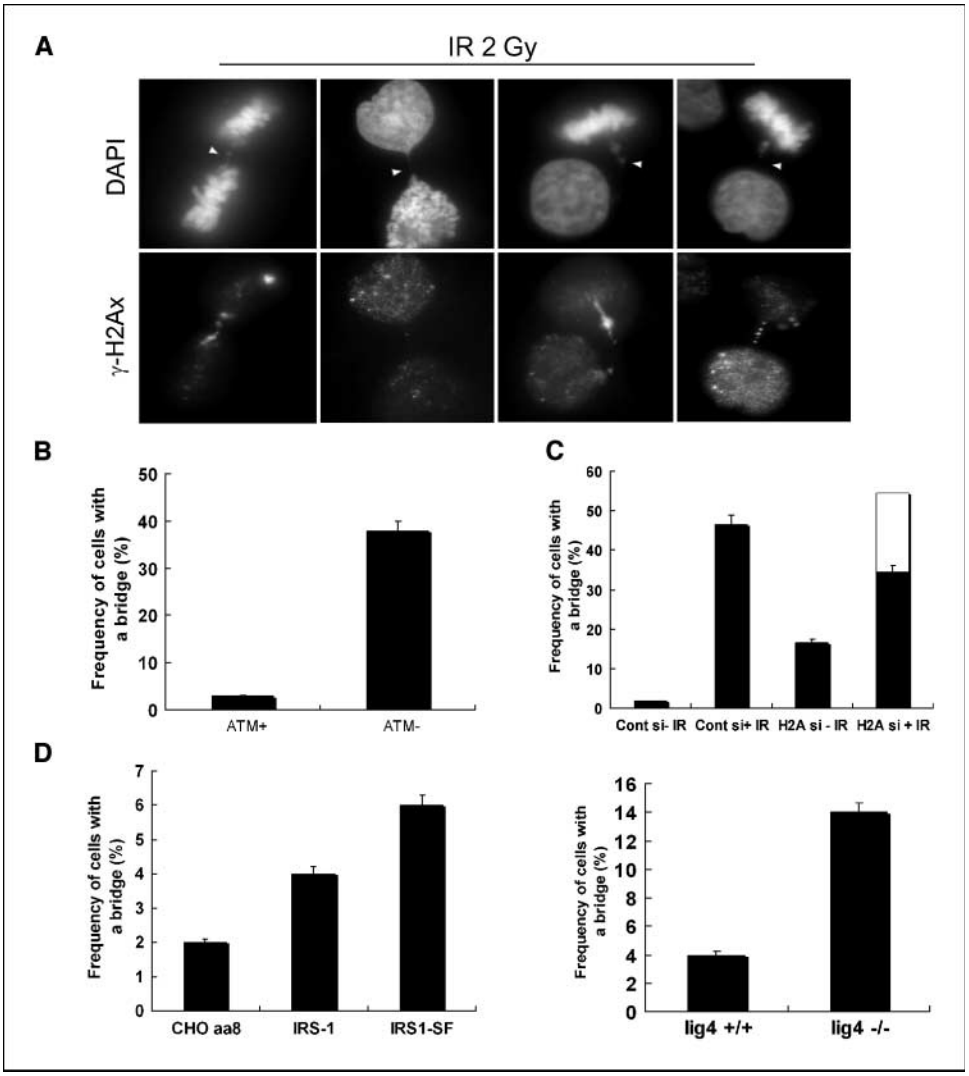

Figure 6. Detection of DNA damage in irradiation-induced bridged cells. *A,* HeLa cells were irradiated with 2 Gy and stained with DAPI and immunostained for phosphorylated γ-H2AX. *B,* AT22IJE (*ATM+*) and AT22IJE-T (*ATM−*) cells were irradiated with 6 Gy of X-rays, fixed and stained with DAPI at 24 h postirradiation. The number of bridged cells were manually counted and plotted as a percentage of the total number of observed cells. *C,* the number of bridged HeLa cells that were transfected with control and H2AX siRNAs was irradiated with 6 Gy and then manually quantitated (n > 300 cells). *White column,* an extrapolation of the number of cells that would have been counted if they did not die in mitosis. *D,* the number of DNA-bridged cells at 24 h after irradiation with 1 Gy for Chinese hamster ovary *aa8, IRS-1*, and *IRS-1 SF* as well as immortalized, *p53−/−* MEFs either wild-type or *ligase 4−/−* were irradiated (1 Gy) and the number of cells connected by a DNA bridge was compared.

are products of damaged and unrepaired DNA. This conclusion is further supported by the findings that disruption of the DNA damage response pathway by inactivation of *ATM* or *H2AX* also led to the increased frequency of chromatin bridges. *BRCA2* deficiency also results in a delayed cytokinesis that has been proposed to be due to mislocalization of myosin IIB from the site of cleavage. The bridged cells examined in that study did not contain detectable DNA and they ascribe a novel function for BRCA2 in organizing the cleavage furrow during cytokinesis (38). *XRCC3* mutant cells have been reported to exhibit elevated frequencies of binucleated and multinucleated cells and apoptosis (39). Whether this is due to defects in DNA repair and chromatin bridge formation remains to be investigated. Finally, Bloom's protein was recently reported to play a role in chromosome segregation by resolving previously unseen chromatin fibers that stretch between separated chromatids in cells that are in anaphase (40). The contribution of this newly identified function of Bloom's protein in resolving the chromatin bridges that accumulate in irradiated cells also remains to be investigated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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