

# Enhanced fidelity for rejoining radiation-induced DNA double-strand breaks in the G<sub>2</sub> phase of Chinese hamster ovary cells

Ines Krüger, Kai Rothkamm and Markus Löbrich\*

Fachrichtung Biophysik, Universität des Saarlandes, D-66421 Homburg/Saar, Germany

Received February 26, 2004; Revised and Accepted April 14, 2004

## ABSTRACT

**The influence of cell cycle phase on the fidelity of DNA double-strand break (DSB) repair is largely unknown. We investigated the rejoining of correct and incorrect DSB ends in synchronized populations of Chinese hamster ovary cells irradiated with 80 Gy X-rays. A specialized pulsed-field gel electrophoresis assay based on quantitative Southern hybridization of individual large restriction fragments was employed to measure correct DSB rejoining by monitoring restriction fragment reconstitution. Total DSB repair, representing both correct and incorrect rejoining, was analyzed using conventional pulsed-field gel electrophoresis. We present evidence that restriction fragment reconstitution is more efficient in G<sub>2</sub> than in G<sub>1</sub>, suggesting that DSB rejoining in G<sub>2</sub> proceeds with higher fidelity. DNA-dependent protein kinase-deficient V3 and xrs-6 cells show impaired restriction fragment reconstitution in G<sub>1</sub> and G<sub>2</sub> compared with wild-type AA8 and K1 cells, demonstrating that the enhanced fidelity of DSB rejoining in G<sub>2</sub> occurs by non-homologous end joining. Additionally, homologous recombination-deficient irs1SF and wild-type cells show identical DSB rejoining in G<sub>1</sub> and G<sub>2</sub>. We propose that structural characteristics of G<sub>2</sub> phase chromatin, such as the cohesion of sister chromatids in replicated chromatin, limit the mobility of radiation-induced break ends and enhance the fidelity of DSB rejoining.**

## INTRODUCTION

Of the many types of damage that arise in DNA, the most critical lesions are DNA double-strand breaks (DSBs). They are produced by ionizing radiation (IR) and certain chemicals and arise endogenously during DNA replication and during developmental processes such as V(D)J recombination and meiotic crossing-over. If left unrepaired, DSBs can induce

permanent cell cycle arrest, apoptosis or mitotic cell death caused by loss of genomic material (1). If repaired incorrectly, they can trigger carcinogenesis through translocations, inversions or deletions of genomic material (2,3).

Higher eukaryotic cells primarily utilize two genetically separable pathways for DSB repair, non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ repairs DSBs without a requirement for extensive sequence homology at the break ends. It involves the XRCC4–DNA ligase IV complex and the DNA-dependent protein kinase (DNA-PK) holoenzyme, which consists of the DNA end-binding heterodimer Ku70/Ku80 and the catalytic subunit DNA-PK<sub>cs</sub> (4–6). Defects in any of these proteins confer IR sensitivity and defective DSB rejoining (7–10). Functional NHEJ has been described as a caretaker of chromosomal integrity (11,12) and increased levels of radiation-induced chromosomal aberrations have been observed in cells deficient in NHEJ (13–16). However, NHEJ has the potential to generate genomic rearrangements by joining incorrect break ends (17,18). The probability for this type of mis-rejoining has been proposed to be influenced by the structural characteristics of the chromatin, which may limit the diffusibility of break ends (19).

HR, which utilizes an undamaged homolog to faithfully restore the sequence at the break site (20), appears to be active predominantly in the S and G<sub>2</sub> phases of the cell cycle, when a sister chromatid is available (21). HR is predicted to rejoin breaks accurately since heteroallelic recombination has been reported to be a very rare event (22) and since the outcome of HR in mammalian cells is predominantly gene conversion without crossing-over (23). Thus, HR should not contribute to genomic rearrangements. We have recently assessed the relative contribution of NHEJ and HR to DSB repair in different cell cycle phases by enumerating  $\gamma$ -H2AX foci after low (1 Gy) X-ray doses (24). We found that NHEJ is important for cell survival and DSB repair throughout the cell cycle whereas HR preferentially contributes to DSB repair and survival in late S/G<sub>2</sub> (24).

We have previously reported that the induction and correct rejoining of DSBs can be analyzed by evaluating large genomic restriction fragments in irradiated and repair-incubated mammalian cells (18,19,25). Our assay to monitor

\*To whom correspondence should be addressed. Tel: +49 6841 1626202; Fax: +49 6841 1626160; Email: markus.loebrich@uniklinik-saarland.de  
Present address:

Kai Rothkamm, Gray Cancer Institute, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, UK

correct DSB rejoining involves restriction enzyme digestion of genomic DNA after irradiation and repair incubation, followed by separation of the restriction fragments using pulsed-field gel electrophoresis (PFGE) and subsequent detection of a specific fragment by Southern hybridization. DSB induction is assessed by a decrease in intensity of the hybridizing restriction fragment band and correct DSB rejoining is monitored by restriction fragment reconstitution. Since rejoining of correct DSB ends (i.e. ends that were linked before irradiation) reconstitutes the original restriction fragment whereas joining of incorrect break ends generates aberrant restriction fragments that are smaller or larger than the original fragment, the rejoining of correct DSB ends, also termed correct DSB rejoining, can be assessed by measuring the extent of reconstitution of the original restriction fragment band. However, in this assay correct rejoining might also include events involving gain or loss of short DNA sequences. Correct DSB rejoining is taken to represent a high fidelity process and is contrasted with the joining of incorrect break ends, also termed mis-rejoining, that includes, for example, translocations or inversions. The aim of this work was to evaluate the fidelity of DSB rejoining in different phases of the mammalian cell cycle.

## MATERIALS AND METHODS

### Cell synchronization, irradiation and lysis

Chinese hamster ovary (CHO) cells (K1, AA8, xrs-6, V3 and irs1SF) were grown in MEM supplemented with 10% fetal bovine serum and antibiotics. Cells were seeded at a density of  $8 \times 10^4$  cells/cm<sup>2</sup> and grown for 3–4 days to obtain G<sub>1</sub> phase cells;  $4 \times 10^4$  G<sub>1</sub> phase cells/cm<sup>2</sup> were grown for 16 h in medium containing 1 µg/ml aphidicolin. Under these conditions, cells accumulate at the G<sub>1</sub>/S border. The aphidicolin-containing medium was then removed and the cells were incubated for 6 h (6.5 h for irs1SF cells) in fresh medium to obtain G<sub>2</sub> phase cells. Measurements of cell cycle distributions were performed with a FACScan flow cytometer and CellQuest software (BD Biosciences, San Diego, CA). Cells were harvested, resuspended in phosphate-buffered saline (PBS), fixed with 70% ethanol at -20°C and stained with propidium iodide/RNase A. X-irradiation was performed at 95 kV and 25 mA with a 1.3 mm aluminum filter and a dose rate of ~6 Gy/min as determined by chemical dosimetry. Cells were irradiated in flasks filled with ice-cold PBS, which was replaced by pre-warmed medium for repair incubation. Control samples were sham-irradiated in all experiments. Cell lysis and DNA extraction were performed as previously described (26). Briefly, cells were harvested, embedded in agarose plugs ( $6 \times 10^5$  G<sub>1</sub> phase cells/plug and  $3 \times 10^5$  G<sub>2</sub> phase cells/plug) and lysed with pronase E at 50°C for 2 days.

### PFGE analysis

To determine total DSB rejoining (FAR assay), DNA was separated by PFGE with a CHEF DR system (Bio-Rad, Hercules, CA) in 0.8% agarose gels. The gels were run at 14°C with linearly increasing pulse times from 50 to 5000 s over 65 h at a field strength of 1.5 V/cm. Gels were stained with ethidium bromide, images were obtained with a charge-coupled device camera under UV illumination and the fraction

of DNA below the well was quantified with the AIDA software (Raytest, Straubenhardt, Germany). Experiments measuring the fraction of DNA below the well as a function of dose were performed in parallel to repair experiments, with the results serving as a calibration to obtain relative numbers of remaining DSBs from the fraction of DNA below the well in the repair samples. To determine restriction fragment reconstitution (i.e. the level of correct DSB rejoining), DNA was digested with the rare-cutting restriction enzyme MluI prior to electrophoresis. Agarose gels (0.8%) were run at 16°C for 46 h at 3 V/cm with pulse times increasing linearly from 40 to 800 s. After PFGE separation, the DNA was partly dephosphorylated and transferred to a nylon membrane (Hybond XL; Amersham Pharmacia) by vacuum alkaline transfer. Pre-hybridization and hybridization were performed in 20 ml of 5× SSPE, 5× Denhardt's, 3% SDS and 100 µg/ml sonicated denatured salmon sperm DNA at 65°C in hybridization bottles. Chinese hamster dihydrofolate reductase cDNA (ATCC, catalog no. 77273), which detects a 1.9 Mb MluI restriction fragment of hamster DNA (26,27), was labeled by random priming with [ $\alpha$ -<sup>32</sup>P]dCTP. Membranes were wrapped in Saran wrap after washing and exposed for several days to an imaging screen. The screen was scanned by a phosphorimaging system and quantitative analysis was carried out using the AIDA software (Raytest). A detailed description of the assays and the evaluation procedures are published in Rothkamm and Löbrich (26).

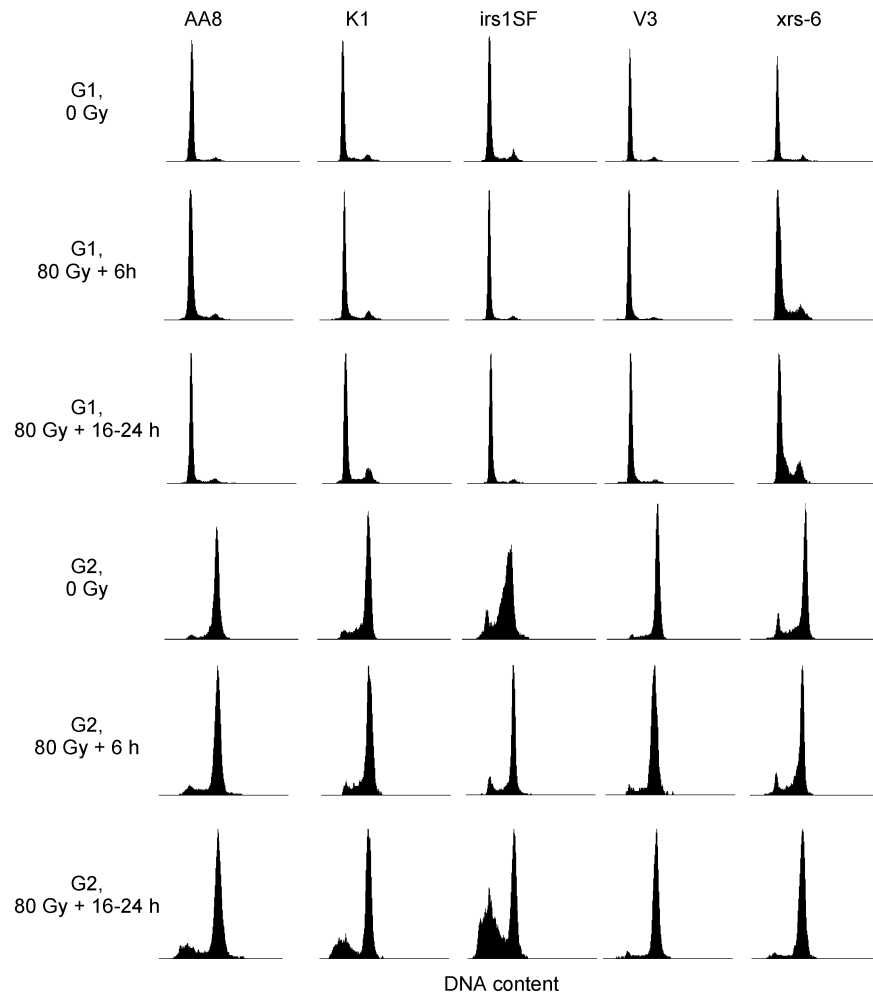
## RESULTS

### Similar yield of X-ray-induced DSBs in G<sub>1</sub> and G<sub>2</sub>

Parental AA8 and K1 cells, XRCC3-defective irs1SF (28), DNA-PK<sub>cs</sub>-defective V3 and Ku80-defective xrs-6 cells (4) were grown to confluence to obtain at least 90% G<sub>1</sub> phase cells (Fig. 1, first row). To investigate DSB induction and rejoining in G<sub>2</sub>, confluent cells were subcultured and treated with aphidicolin for 16 h. Six hours (6.5 h for irs1SF cells) after release from the aphidicolin block, >80% of the cells were in late S/G<sub>2</sub> (Fig. 1, fourth row). DSB induction was measured with the specialized PFGE assay described above by quantifying the intensity of the hybridization signal representing the intact restriction fragment ( $I_{RF}$ ), which is normalized to the total signal intensity of each lane ( $I_T$ ) to account for differences in DNA loading. After radiation exposure,  $I_{RF}$  decreases in a dose-dependent manner (see Fig. 4, lanes 1–5). In this approach, the number of DSBs induced per restriction fragment equals the negative logarithm of the ratio of  $I_{RF}/I_T$  of the irradiated sample to  $I_{RF}/I_T$  of the control sample (26). In all cell lines, the yield of DSB induction (expressed as DSBs/Mb/Gy) was very similar for G<sub>1</sub> and G<sub>2</sub> phase cells (Fig. 2), indicating that DNA is equally susceptible to radiation-induced breakage in both cell cycle phases.

### Improved DSB rejoining in G<sub>2</sub> phase NHEJ mutants

Conventional PFGE analysis was applied to analyze the time course for total DSB rejoining after 80 Gy X-rays. AA8, K1 and the HR mutant line irs1SF repair DSBs with similar kinetics in G<sub>1</sub> and G<sub>2</sub> (Fig. 3). After a repair period of 24 h, nearly all DSBs are rejoined in all cell lines, indicating that DSB rejoining is very efficient in the absence of HR in both G<sub>1</sub>



**Figure 1.** DNA histograms of unirradiated and 80 Gy irradiated CHO cells synchronized in G<sub>1</sub> (upper three rows) and G<sub>2</sub> (lower three rows).

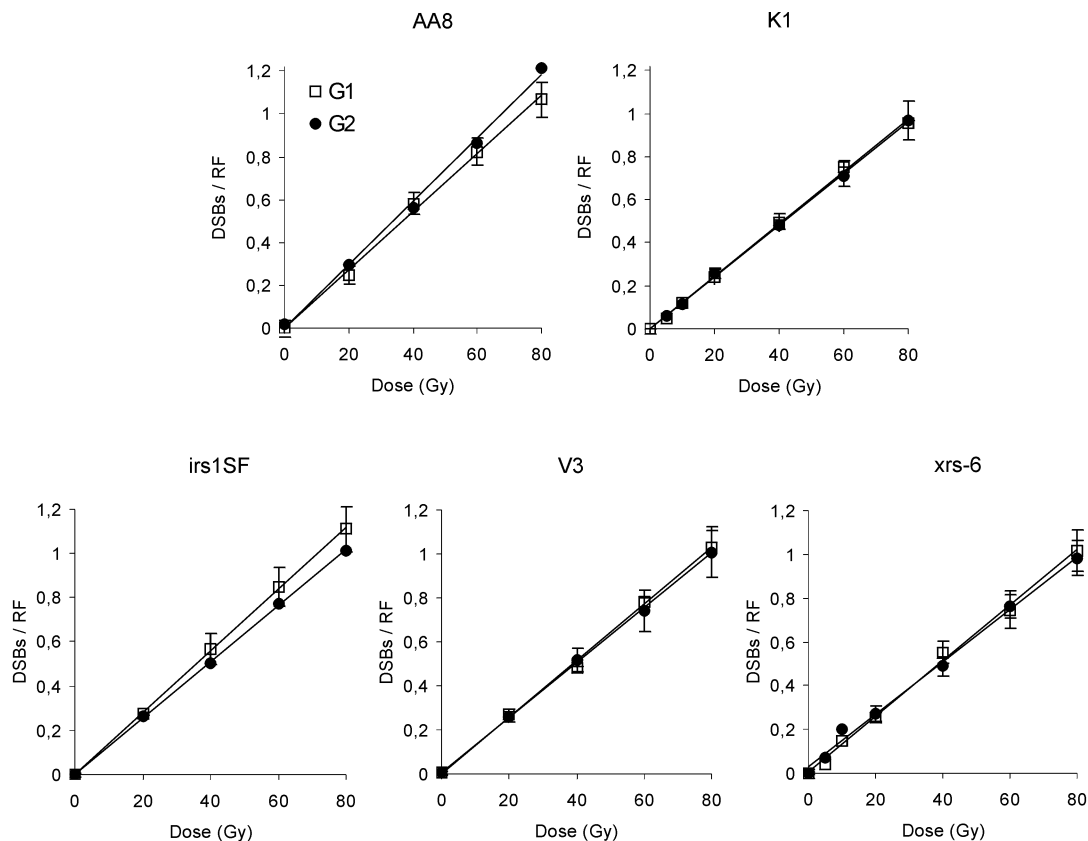
and G<sub>2</sub>. The NHEJ mutants xrs-6 and V3, in contrast, exhibit a pronounced repair defect with ~40% unrejoined DSBs in G<sub>1</sub> (Fig. 3), consistent with previous measurements (18). Interestingly, only 10–20% unrejoined DSBs are observed in xrs-6 and V3 cells in G<sub>2</sub>, indicating that specific factors or conditions enhance the capacity for DSB rejoining in this cell cycle phase. This result is consistent with data from  $\gamma$ -H2AX analysis, which also indicate substantially improved DSB rejoining in G<sub>2</sub> phase NHEJ mutants (24). Compared with wild-type cells and HR mutants, however, NHEJ-deficient cells exhibit impaired kinetics in G<sub>2</sub> (Fig. 3).

#### Restriction fragment reconstitution is more efficient in G<sub>2</sub> than in G<sub>1</sub>

To investigate the fidelity of DSB rejoining in G<sub>1</sub> and G<sub>2</sub>, the specialized PFGE approach was employed. For a quantitative analysis, the band intensity,  $I_{RF}$ , of a repair sample is transformed into the number of DSBs (by taking the negative logarithm of the ratio of  $I_{RF}/I_T$  of the repair sample to  $I_{RF}/I_T$  of the control sample; see above) and this number is divided by the number of DSBs obtained in a sample irradiated with the same dose but not incubated for repair. This provides an estimate of the fraction of DSBs that have not been rejoined correctly (i.e. DSBs that have either remained unrejoined or

have been mis-rejoined to generate an aberrant restriction fragment). Analysis of the time course for correct DSB rejoining after 80 Gy shows that repair-proficient AA8 and K1 cells in G<sub>1</sub> rejoin 50–60% of the induced DSBs correctly (Fig. 4), consistent with previous observations in wild-type CHO cells (18,26,27). For the same cells synchronized in G<sub>2</sub>, 80–90% correct rejoining is observed. This indicates that the fidelity of DSB rejoining is significantly higher in G<sub>2</sub> than in G<sub>1</sub>.

We next investigated the fidelity of DSB rejoining in irs1SF, V3 and xrs-6 cells (Fig. 4). Importantly, in G<sub>1</sub> as well as in G<sub>2</sub>, the kinetics of correct DSB rejoining for irs1SF cells are very similar to those found with wild-type cells, indicating that HR does not contribute significantly to correct DSB rejoining and is not responsible for the enhanced fidelity of DSB rejoining in G<sub>2</sub> phase wild-type cells. Examination of the time course for restriction fragment reconstitution in NHEJ-deficient cells shows significantly improved band reconstitution in G<sub>2</sub> compared with G<sub>1</sub>, similar to the result for total DSB rejoining obtained by conventional PFGE analysis. Moreover, in both cell cycle phases restriction fragment reconstitution is less efficient in the NHEJ mutants than in wild-type cells, indicating that NHEJ contributes to correct DSB rejoining in both G<sub>1</sub> and G<sub>2</sub>.



**Figure 2.** Yields of DSBs as a function of radiation dose for G<sub>1</sub> and G<sub>2</sub> phase CHO cells as determined by Southern hybridization analysis of a 1.9 Mb restriction fragment (RF). Average induction rates are as follows (in 10<sup>-3</sup> DSBs/Mb/Gy): AA8, G<sub>1</sub> 7.2, G<sub>2</sub> 7.8; K1, G<sub>1</sub> 6.4, G<sub>2</sub> 6.4; irs1SF, G<sub>1</sub> 7.4, G<sub>2</sub> 6.7; V3, G<sub>1</sub> 6.7, G<sub>2</sub> 6.6; xrs-6, G<sub>1</sub> 6.7, G<sub>2</sub> 6.3. Error bars represent the SEM from two or three experiments.

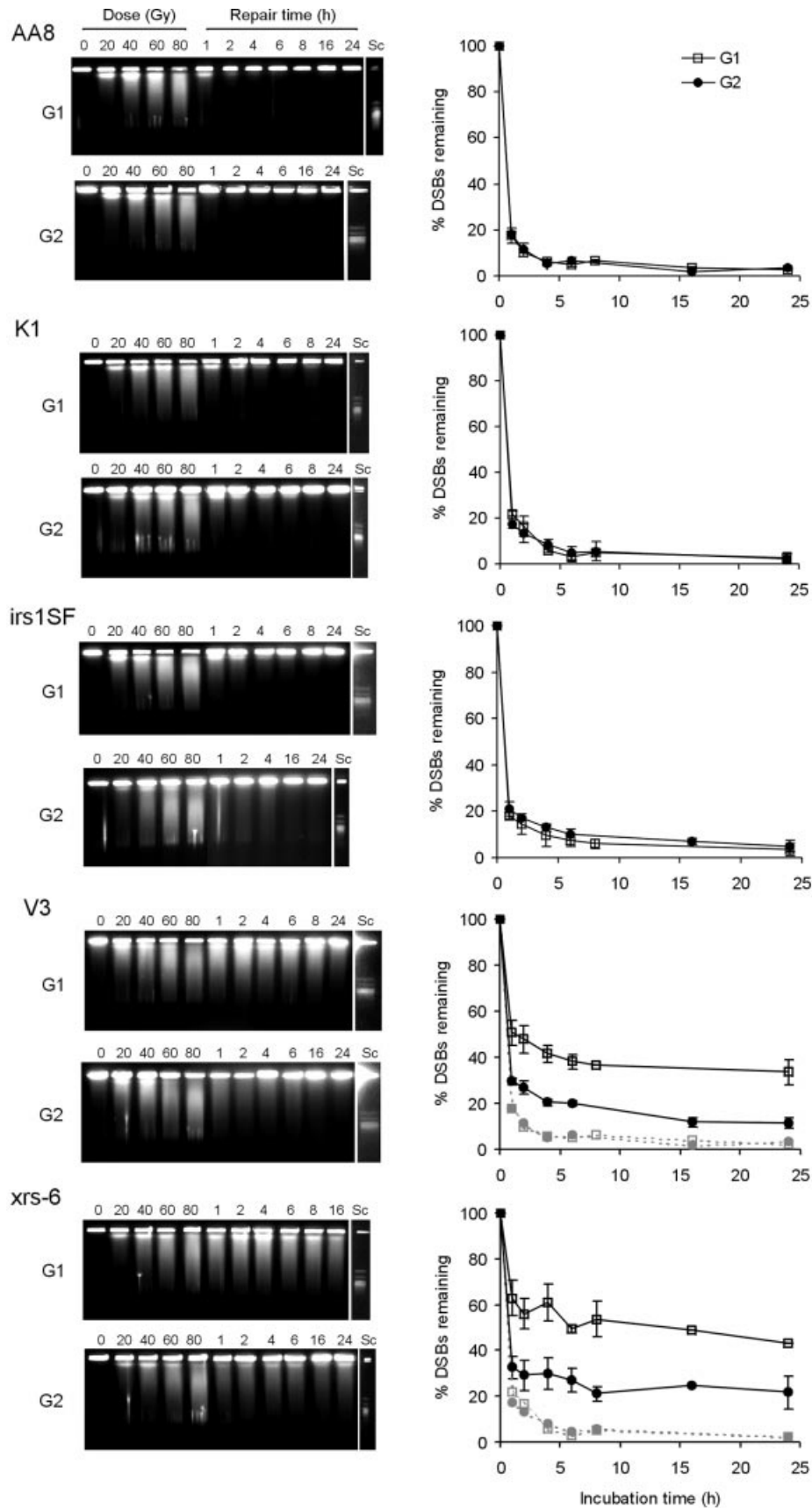
## DISCUSSION

We employed two different PFGE techniques to investigate DSB repair in different phases of the mammalian cell cycle. Conventional PFGE was employed to measure total DSB rejoining that encompasses both correct and incorrect rejoining events while a specialized PFGE approach was used to assess the induction and correct rejoining of DSBs. Using a similar strategy, we previously showed that NHEJ can effect incorrect DSB rejoining following high acute radiation doses (18) and that the extent of incorrect rejoining is lower in heterochromatic centromere regions than in average genomic DNA (19). Here, we analyze DSB rejoining in the G<sub>1</sub> and G<sub>2</sub> phases of wild-type CHO cells after 80 Gy X-irradiation. Consistent with published data (29,30), we observed a similar time course for total DSB rejoining in both phases of the cell cycle (Fig. 3). In contrast, correct DSB rejoining is substantially higher in G<sub>2</sub> compared with G<sub>1</sub> (Fig. 4). This finding indicates that G<sub>2</sub>-specific conditions, although not contributing to the time course of total DSB rejoining, enhance the fidelity of the rejoining process. We tested the possibility that HR contributes to this effect and did not observe any significant DSB repair defect in HR-deficient irs1SF cells in late S/G<sub>2</sub> using either the conventional or the specialized PFGE technique. This finding is consistent with previous reports that HR-defective cells fail to exhibit a DSB repair defect in assays that require high radiation doses (31–34) but

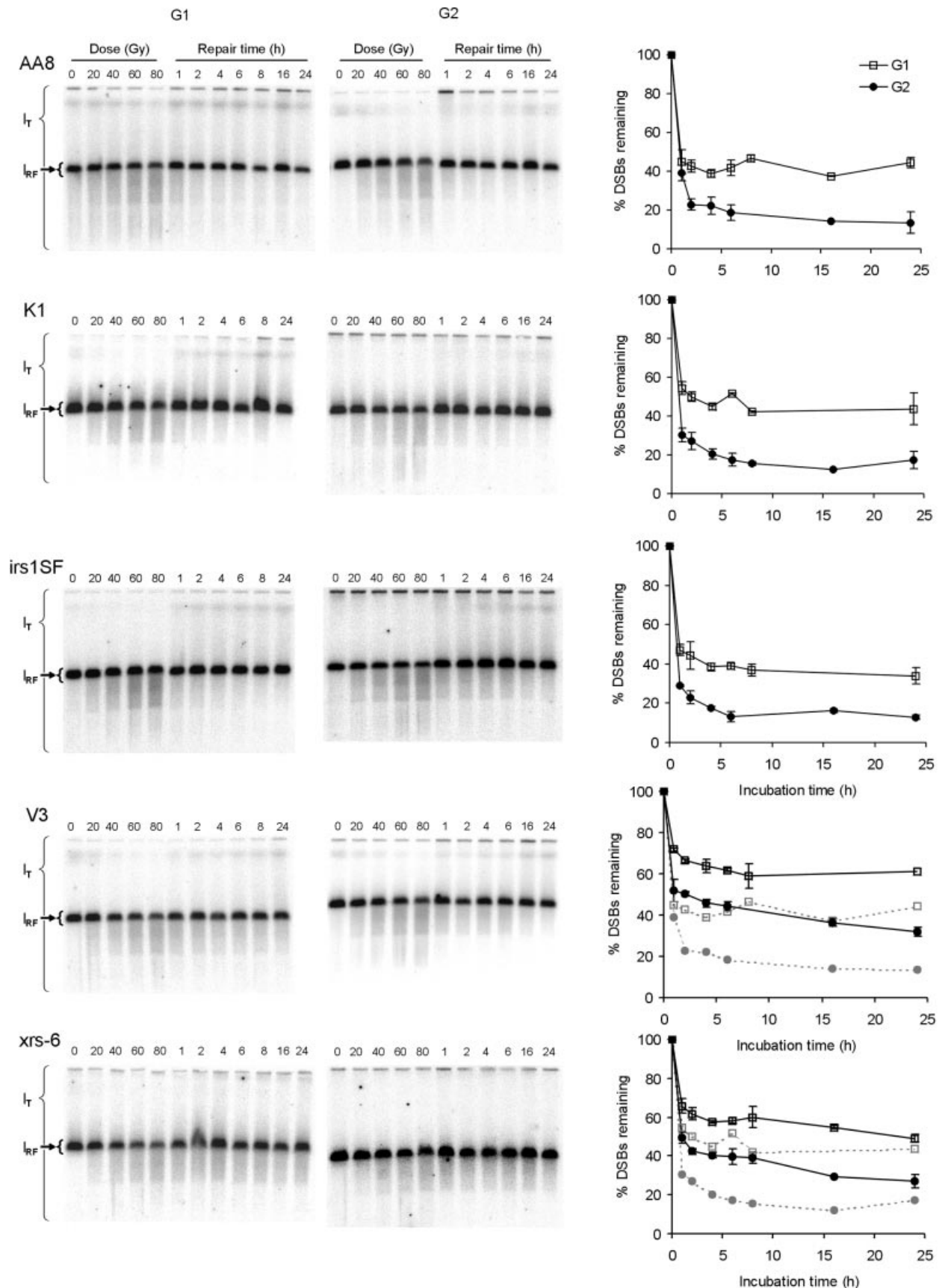
contrasts with results from  $\gamma$ -H2AX analysis after a dose of 1 Gy that uncovered a role of HR in DSB repair in G<sub>2</sub> (24). It is possible that PFGE fails to uncover this contribution because of the necessity of using high doses and/or the limited sensitivity of this technique. However, even after a dose of 1 Gy, only a few DSBs remain unrepaired in HR-deficient cells. Hence, both techniques (PFGE at high doses and  $\gamma$ -H2AX analysis at low doses) demonstrate that the majority of the rejoining events in G<sub>2</sub> involve HR-independent processes (24).

Our data demonstrate that HR-independent processes rejoin DSBs with higher fidelity in G<sub>2</sub> than in G<sub>1</sub>. The observation that NHEJ mutants exhibit impaired restriction fragment reconstitution in G<sub>1</sub> and G<sub>2</sub> demonstrates the importance of this pathway for correct DSB rejoining in both cell cycle phases. Additionally, DSB rejoining in the absence of DNA-PK proceeds with faster kinetics and higher fidelity in G<sub>2</sub> than in G<sub>1</sub>. This finding is consistent with earlier studies with synchronized populations of NHEJ-deficient cells that show increased DSB rejoining in NHEJ mutants in G<sub>2</sub> relative to G<sub>1</sub> (35,36). Substantial DSB rejoining is also observed after 20 and 80 Gy in G<sub>2</sub> phase populations of DT40 chicken cells deficient for both Ku70 and Rad54 (34; K. Rothkamm and M. Löbrich, unpublished data), consistent with the idea that DSBs can be rejoined in the absence of these factors (37,38).

A possible explanation for the increased fidelity of DSB rejoining in G<sub>2</sub> involves structural differences between G<sub>1</sub> and



**Figure 3.** DSB repair in G<sub>1</sub> and G<sub>2</sub> phase cells as determined by conventional PFGE. (Left) Ethidium bromide images of DNA from G<sub>1</sub> and G<sub>2</sub> phase cells irradiated with 0, 20, 40, 60 and 80 Gy without repair or incubated for up to 24 h for repair after 80 Gy irradiation. Sc, *Saccharomyces cerevisiae* size standard. (Right) Time course for the percentage of initial DSBs remaining after repair. Error bars represent the SEM from two or three experiments. The data from the top two panels are redrawn in the bottom two panels in grey symbols for a direct assessment of the impact of NHEJ deficiency.



**Figure 4.** Restriction fragment reconstitution in G<sub>1</sub> and G<sub>2</sub> phase cells as determined by Southern hybridization analysis. (Left) Southern hybridization images of MluI-digested DNA from G<sub>1</sub> and G<sub>2</sub> phase cells irradiated with 0, 20, 40, 60 and 80 Gy without repair or incubated for up to 24 h for repair after 80 Gy irradiation. (Right) Time course for the percentage of initial DSBs not correctly rejoined after repair. Error bars represent the SEM from two or three experiments. The data from the top two panels are redrawn in the bottom two panels in grey symbols for a direct assessment of the impact of NHEJ deficiency.

G<sub>2</sub> phase chromatin. Such differences could be effected by the cohesin complex, which consists of the heterodimer SMC1 and SMC3 and at least two non-SMC subunits (Scc1/Mcd1/RAD21 and Scc3/SAs). In *Saccharomyces cerevisiae*, cohesin associates with specific regions near centromeres and along chromosome arms with a preference for AT-rich sequences. In the arms, cohesin distributes with a periodicity of 9–15 kb (39). Throughout G<sub>2</sub>, sister chromatid cohesion may prevent free break ends from long-range diffusion, thereby promoting post-replicative repair processes (40) until cohesin is unloaded at the onset of anaphase. Our data support a model in which radiation-induced DSBs are less diffusible in G<sub>2</sub> cells due to sister chromatid cohesion, resulting in a reduced probability of end joining leading to the joining of incorrect break ends. This model is consistent with results from a recent study in our group which demonstrated enhanced restriction fragment reconstitution in highly condensed centromeric DNA in comparison to euchromatic DNA (19). It is worth noting that the G<sub>1</sub> phase populations used in the present study were obtained by growing cells to confluency. Confluent, non-growing G<sub>1</sub> cells may have exited the cell cycle and possibly could possess a state of chromatin condensation different from growing G<sub>1</sub> cells. It is also interesting that G<sub>2</sub> phase and non-growing G<sub>1</sub> phase CHO cells show approximately the same level of radiosensitivity (24), consistent with our previous notion that DSB mis-rejoining may not necessarily be a lethal event and more likely may have consequences for other biological end-points such as mutations and chromosome exchange aberrations (41).

## ACKNOWLEDGEMENTS

We would like to thank P. Jeggo for providing AA8 and V3 cells, R. Greinert for sending K1 cells, L. Thompson for providing irs1SF cells, A. Krempler and P. Jeggo for comments on the manuscript and M. Kühne for help in preparing figures. xrs-6 cells (European Collection of Cell Cultures) are commercially available. Financial support was provided by the Deutsche Forschungsgemeinschaft (grants Lo 677/1-1 and Lo 677/1-2) and the Radiation Protection Programme of the European Community (FIGH-CT-1999-00012).

## REFERENCES

- Olive, P.L. (1998) The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. *Radiat. Res.*, **150**, S42–S51.
- Hoeijmakers, J.H. (2001) Genome maintenance mechanisms for preventing cancer. *Nature*, **411**, 366–374.
- van Gent, D.C., Hoeijmakers, J.H. and Kanaar, R. (2001) Chromosomal stability and the DNA double-stranded break connection. *Nature Rev. Genet.*, **2**, 196–206.
- Jeggo, P.A. (1998) DNA breakage and repair. In Hall, J.C., Dunlap, J.C., Friedmann, T. and Gianelli, F. (eds), *Advances in Genetics*. Academic Press, San Diego, CA, Vol. 38, pp. 185–218.
- Smith, G.C. and Jackson, S.P. (1999) The DNA-dependent protein kinase. *Genes Dev.*, **13**, 916–934.
- Jackson, S.P. (2002) Sensing and repairing DNA double-strand breaks. *Carcinogenesis*, **23**, 687–696.
- Chang, C., Biedermann, K.A., Mezzina, M. and Brown, J.M. (1993) Characterization of the DNA double strand break repair defect in scid mice. *Cancer Res.*, **53**, 1244–1248.
- Kurimasa, A., Kumano, S., Boubnov, N.V., Story, M.D., Tung, C.S., Peterson, S.R. and Chen, D.J. (1999) Requirement for the kinase activity of human DNA-dependent protein kinase catalytic subunit in DNA strand break rejoining. *Mol. Cell. Biol.*, **19**, 3877–3884.
- Riballo, E., Critchlow, S.E., Teo, S.H., Doherty, A.J., Priestley, A., Broughton, B., Kysela, B., Beamish, H., Plowman, N., Arlett, C.F., Lehmann, A.R., Jackson, S.P. and Jeggo, P.A. (1999) Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr. Biol.*, **9**, 699–702.
- Wachsberger, P.R., Li, W.H., Guo, M., Chen, D., Cheong, N., Ling, C.C., Li, G. and Iliakis, G. (1999) Rejoining of DNA double-strand breaks in Ku80-deficient mouse fibroblasts. *Radiat. Res.*, **151**, 398–407.
- Difilippantonio, M.J., Zhu, J., Chen, H.T., Meffre, E., Nussenzweig, M.C., Max, E.E., Ried, T. and Nussenzweig, A. (2000) DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature*, **404**, 510–514.
- Gao, Y., Ferguson, D.O., Xie, W., Manis, J.P., Sekiguchi, J., Frank, K.M., Chaudhuri, J., Horner, J., DePinho, R.A. and Alt, F.W. (2000) Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature*, **404**, 897–900.
- Darroudi, F. and Natarajan, A.T. (1987) Cytological characterization of Chinese hamster ovary X-ray-sensitive mutant cells, xrs 5 and xrs 6. II. Induction of sister-chromatid exchanges and chromosomal aberrations by X-rays and UV-irradiation and their modulation by inhibitors of poly(ADP-ribose) synthetase and alpha-polymerase. *Mutat. Res.*, **177**, 149–160.
- Evans, J.W., Liu, X.F., Kirchgessner, C.U. and Brown, J.M. (1996) Induction and repair of chromosome aberrations in scid cells measured by premature chromosome condensation. *Radiat. Res.*, **145**, 39–46.
- Iliakis, G.E. and Pantelias, G.E. (1990) Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analysed in interphase using the technique of premature chromosome condensation. *Int. J. Radiat. Biol.*, **57**, 1213–1223.
- Kemp, L.M. and Jeggo, P.A. (1986) Radiation-induced chromosome damage in X-ray-sensitive mutants (xrs) of the Chinese hamster ovary cell line. *Mutat. Res.*, **166**, 255–263.
- Richardson, C. and Jasin, M. (2000) Frequent chromosomal translocations induced by DNA double-strand breaks. *Nature*, **405**, 697–700.
- Rothkamm, K., Kühne, M., Jeggo, P.A. and Löbrich, M. (2001) Radiation-induced genomic rearrangements formed by nonhomologous end-joining of DNA double-strand breaks. *Cancer Res.*, **61**, 3886–3893.
- Rief, N. and Löbrich, M. (2002) Efficient rejoining of radiation-induced DNA double-strand breaks in centromeric DNA of human cells. *J. Biol. Chem.*, **277**, 20572–20582.
- Thompson, L.H. and Schild, D. (2002) Recombinational DNA repair and human disease. *Mutat. Res.*, **509**, 49–78.
- Thacker, J. (1999) A surfeit of RAD51-like genes? *Trends Genet.*, **15**, 166–168.
- Sonoda, E., Takata, M., Yamashita, Y.M., Morrison, C. and Takeda, S. (2001) Homologous DNA recombination in vertebrate cells. *Proc. Natl Acad. Sci. USA*, **98**, 8388–8394.
- Johnson, R.D. and Jasin, M. (2001) Double-strand-break-induced homologous recombination in mammalian cells. *Biochem. Soc. Trans.*, **29**, 196–201.
- Rothkamm, K., Krüger, I., Thompson, L.H. and Löbrich, M. (2003) Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol. Cell. Biol.*, **23**, 5706–5715.
- Löbrich, M., Rydberg, B. and Cooper, P.K. (1995) Repair of x-ray-induced DNA double-strand breaks in specific NotI restriction fragments in human fibroblasts: joining of correct and incorrect ends. *Proc. Natl Acad. Sci. USA*, **92**, 12050–12054.
- Rothkamm, K. and Löbrich, M. (1999) Misrejoining of DNA double-strand breaks in primary and transformed human and rodent cells: a comparison between the HPRT region and other genomic locations. *Mutat. Res.*, **433**, 193–205.
- Fouladi, B., Waldren, C.A., Rydberg, B. and Cooper, P.K. (2000) Comparison of repair of DNA double-strand breaks in identical sequences in primary human fibroblast and immortal hamster-human hybrid cells harboring a single copy of human chromosome 11. *Radiat. Res.*, **153**, 795–804.
- Liu, N., Lamerdin, J.E., Tebbs, R.S., Schild, D., Tucker, J.D., Shen, M.R., Brookman, K.W., Siciliano, M.J., Walter, C.A., Fan, W., Narayana, L.S., Zhou, Z.Q., Adamson, A.W., Sorensen, K.J., Chen, D.J., Jones, N.J. and Thompson, L.H. (1998) XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell*, **1**, 783–793.

29. Iliakis,G.E., Cicilioni,O. and Metzger,L. (1991) Measurement of DNA double-strand breaks in CHO cells at various stages of the cell cycle using pulsed field gel electrophoresis: calibration by means of <sup>125</sup>I decay. *Int. J. Radiat. Biol.*, **59**, 343–357.
30. Metzger,L. and Iliakis,G. (1991) Kinetics of DNA double-strand break repair throughout the cell cycle as assayed by pulsed field gel electrophoresis in CHO cells. *Int. J. Radiat. Biol.*, **59**, 1325–1339.
31. Jones,N.J., Stewart,S.A. and Thompson,L.H. (1990) Biochemical and genetic analysis of the Chinese hamster mutants *irs1* and *irs2* and their comparison to cultured ataxia telangiectasia cells. *Mutagenesis*, **5**, 15–23.
32. Thacker,J. and Ganesh,A.N. (1990) DNA-break repair, radioresistance of DNA synthesis and camptothecin sensitivity in the radiation-sensitive *irs* mutants: comparisons to ataxia-telangiectasia cells. *Mutat. Res.*, **235**, 49–58.
33. Cheong,N., Wang,Y., Jackson,M. and Iliakis,G. (1992) Radiation-sensitive *irs* mutants rejoin DNA double-strand breaks with efficiency similar to that of parental V79 cells but show altered response to radiation-induced G2 delay. *Mutat. Res.*, **274**, 111–122.
34. Wang,H., Zeng,Z.C., Bui,T.A., Sonoda,E., Takata,M., Takeda,S. and Iliakis,G. (2001) Efficient rejoining of radiation-induced DNA double-strand breaks in vertebrate cells deficient in genes of the RAD52 epistasis group. *Oncogene*, **20**, 2212–2224.
35. Mateos,S., Slijepcevic,P., MacLeod,R.A. and Bryant,P.E. (1994) DNA double-strand break rejoining in *xrs5* cells is more rapid in the G2 than in the G1 phase of the cell cycle. *Mutat. Res.*, **315**, 181–187.
36. Lee,S.E., Mitchell,R.A., Cheng,A. and Hendrickson,E.A. (1997) Evidence for DNA-PK-dependent and -independent DNA double-strand break repair pathways in mammalian cells as a function of the cell cycle. *Mol. Cell. Biol.*, **17**, 1425–1433.
37. DiBiase,S.J., Zeng,Z.C., Chen,R., Hyslop,T., Curran,W.J., Jr and Iliakis,G. (2000) DNA-dependent protein kinase stimulates an independently active, nonhomologous, end-joining apparatus. *Cancer Res.*, **60**, 1245–1253.
38. Wang,H., Perrault,A.R., Takeda,Y., Qin,W., Wang,H. and Iliakis,G. (2003) Biochemical evidence for Ku-independent backup pathways of NHEJ. *Nucleic Acids Res.*, **31**, 5377–5388.
39. Hirano,T. (2002) The ABCs of SMC proteins: two-armed ATPases for chromosome condensation, cohesion and repair. *Genes Dev.*, **16**, 399–414.
40. Sjogren,C. and Nasmyth,K. (2001) Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*. *Curr. Biol.*, **11**, 991–995.
41. Kühne,M., Riballo,E., Rief,N., Rothkamm,K., Jeggo,P.A. and Löbrich,M. (2004) A double-strand break repair defect in ATM-deficient cells contributes to radiosensitivity. *Cancer Res.*, **64**, 500–508.