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DNA damage induces Chk1-dependent centrosome amplification

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Centrosomal abnormalities are frequently observed in cancers and in cells with defective DNA repair. Here, we used light and electron microscopy to show that DNA damage induces centrosome amplification, not fragmentation, in human cells. Caffeine abrogated this amplification in both ATM (ataxia telangiectasia, mutated)- and ATR (ATM and Rad3-related)-defective cells, indicating a complementary role for these DNA-damage-responsive kinases in promoting centrosome amplification. Inhibition of checkpoint kinase 1 (Chk1) by RNA-mediated interference or drug treatment suppressed DNA-damage-induced centrosome amplification. Radiation-induced centrosome amplification was abrogated in *Chk1*$^{-/-}$ DT40 cells, but occurred at normal levels in *Chk1*$^{-/-}$ cells transgenically expressing *Chk1*. Expression of kinase-dead Chk1, or Chk1S345A, through which the phosphatidylinositol-3-kinase cannot signal, failed to restore centrosome amplification, showing that signalling to Chk1 and Chk1 catalytic activity are necessary to promote centrosome overduplication after DNA damage.

Keywords: centrosome; ATM; ATR; Chk1; checkpoint

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INTRODUCTION

Recent findings that link aberrant centrosome number and chromosome instability (Lingle *et al*, 2002) have refocused attention on Boveri's idea that centrosomal defects contribute to tumorigenesis (Brinkley, 2001; Nigg, 2002). There are several models that explain centrosome amplification. Numerical centrosome aberrations can arise after failed cytokinesis (Meraldi *et al*, 2002). Extended G1–S arrest can decouple the centrosome and chromosome cycles in hamster (Balczon *et al*, 1995; Meraldi *et al*, 1999), human (Wong & Stearns, 2003) and chicken (Dodson *et al*, 2004) cells. From the frequent centrosome abnormalities seen in p53-deficient cells (Fukasawa *et al*, 1996), in cells with DNA repair deficiencies (Griffin *et al*, 2000; Dodson *et al*, 2004) and in cells with telomere defects (Guiducci *et al*, 2001), we have suggested that DNA damage signals have an impact on the centrosome during an extended G2–M arrest (Dodson *et al*, 2004).

The principal signalling molecules involved in the DNA damage response are the large serine–threonine kinases of the phosphatidylinositol-3-kinase (PI3K) family—ATM (ataxia telangiectasia, mutated) and ATR (ATM and Rad3-related) (Shiloh, 2003). ATM responds primarily to DNA double-strand breaks (DSBs), whereas ATR acts mainly in response to replication fork stalling, although ionizing radiation also activates ATR. Recent work has shown that ATR activity after DSB induction depends on ATM (Jazayeri *et al*, 2006). The checkpoint kinase 1 (Chk1) is activated by ATM and ATR and is necessary for cell-cycle checkpoints (Bartek & Lukas, 2003). Chk1 is essential in mammalian cells (Liu *et al*, 2000; Takai *et al*, 2000). Replication defects and spontaneous DSBs in Chk1-deficient human and chicken cells indicate that Chk1 might be important in the control of DNA replication (Syljuasen *et al*, 2005; Zachos *et al*, 2005). Loss of Chk1 function in mammalian and chicken cells also disrupts the G2–M checkpoint, giving rise to frequent aberrant mitoses after genotoxic stresses that might also affect cell viability (Takai *et al*, 2000; Zhao *et al*, 2002; Zachos *et al*, 2003).

RESULTS AND DISCUSSION

Early work showed the amplification of microtubule-organizing centres (MTOCs) after irradiation of mouse cells. Electron microscopy showed that a high percentage of these MTOCs did not contain the paired centrioles and pericentrosomal material of the normal centrosome (Sato *et al*, 1983). Later work on irradiated human U2OS cells (an osteosarcoma cell line) demonstrated MTOC amplification after irradiation (Sato *et al*, 2000), although the structure of these MTOCs was not determined. We tested whether human lymphoblastoid cells undergo centrosome amplification after γ-irradiation. As shown in Fig 1A,B, we observed a dose-dependent increase in the number of γ-tubulin foci after ionizing radiation treatment. More than two γ-tubulin foci were

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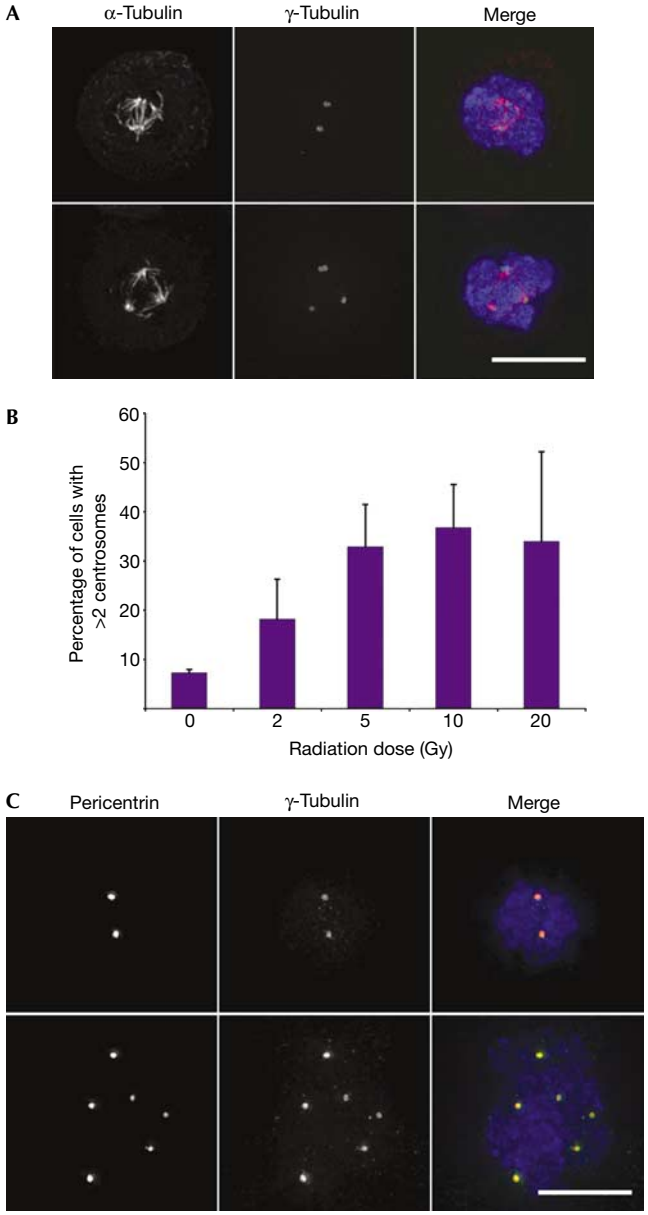

Fig 1 | DNA-damage-induced centrosome amplification in human cells. Cells shown were fixed and stained 48 h after 10 Gy ionizing radiation treatment. DNA counterstains (DAPI) are shown in blue. Scale bars, 10 μm. (**A**) Upper series shows a cell with two centrosomes, and lower series a cell with multiple centrosomes. Cells were stained for γ-tubulin (green) and α-tubulin (red). (**B**) Dose dependence of centrosome amplification in human lymphoblastoid cells 48 h after treatment with the indicated dose of ionizing radiation. The histogram shows mean + s.d. of three separate experiments in which at least 500 cells were analysed. (**C**) The upper series shows a cell with two centrosomes, and the lower series a cell with multiple centrosomes. Cells were stained for pericentrin (red) and γ-tubulin (green). DAPI, 4,6-diamidino-2-phenylindole.

observed in 12% of cells at 24 h after irradiation, compared with 37% at 48 h after 10 Gy treatment. High levels of cell death precluded analysis at 72 h after ionizing radiation. We observed similar amplification in U2OS cells (see later), Hct116 colon carcinoma cells and in Jurkat T-cell lymphoma cells (data not shown). These γ-tubulin foci also contained pericentrin (Fig 1C), indicating that they were indeed centrosomes.

As non-centrosomal aggregation of γ-tubulin or centrosome fragmentation can occur after various treatments (Keryer *et al*, 1984; Hut *et al*, 2003), we carried out serial-section electron microscopy 48 h after 10 Gy irradiation of human lymphoblastoid cells. Cells on glass coverslips were irradiated, then fixed and stained for pericentrin. Cells with multiple pericentrin signals were photographed and, after embedding and relocation, serially sectioned for electron microscopy. We examined three irradiated control cells with multiple pericentrin spots and observed a total of 31 centrioles, of which 18 were clearly paired (Fig 2Ai,ii). In a similar experiment analysing two A-T cells, we observed 15 centrioles, of which 10 were clearly paired (Fig 2Aiii,iv). As a given section might not contain both centrioles of a centrosome, the number of paired centrioles we describe here is the minimum. These findings show that the additional MTOCs that we observed contained duplicated centrosomes rather than fragments. Next, we used immunofluorescence microscopy to verify the composition of the pericentrin/γ-tubulin structures. As shown in Fig 2B, we found that the centrosome components Aurora A, Cep170 (centrosomal protein 170), Nedd1 (neural precursor cell expressed, developmentally downregulated 1), ninein and PCM1 (pericentriolar material 1) showed typical centrosomal localizations (supplementary Fig S1 online; Dammermann & Merdes, 2002; Meraldi *et al*, 2002; Guarguaglini *et al*, 2005). We conclude that centrosome duplication, rather than centrosome splitting or fragmentation, occurs after irradiation.

To define what elements of the DNA damage response regulate centrosome amplification after irradiation of human cells, we examined lymphoblastoid cells with defects in ATM and ATR. The point mutation in *ATR* in Seckel syndrome cells causes a reduction in the protein level, but not its complete loss (O'Driscoll *et al*, 2003). Centrosome amplification after irradiation was observed in A-T cells by using electron microscopy (Fig 2Aiii,iv) and was quantitated by light microscopy with γ-tubulin as a marker (Fig 3B). DNA-damage-induced centrosome amplification was also seen in Seckel syndrome cells (Fig 3B). Normal localization of Aurora A, Cep170, Nedd1, ninein, PCM1 and pericentrin was observed in amplified centrosomes in A-T and Seckel syndrome cells (data not shown). This amplification was dose- and time-dependent in both lymphoblastoid cell lines, and similar actual numbers of centrosomes were seen in wild-type, A-T and Seckel syndrome cells (data not shown). These findings show that neither ATM nor ATR is essential for centrosome amplification.

Next, we used pharmacological means to probe the pathways by which DNA damage causes centrosome amplification. Caffeine is an *in vitro* inhibitor of the ATM/ATR kinases (Sarkaria *et al*, 1999). As shown in Fig 3A, 2 mM caffeine treatment suppressed the G2-phase checkpoint normally observed in human lymphoblastoid cells after irradiation. Caffeine treatment resulted in near-normal numbers of centrosomes after irradiation of control, A-T and Seckel syndrome cells (Fig 3B). That a caffeine-sensitive activity allows centrosome amplification in both ATM- and ATR-deficient cells indicated that they might act in a complementary or redundant manner in driving centrosomal responses to DNA damage.

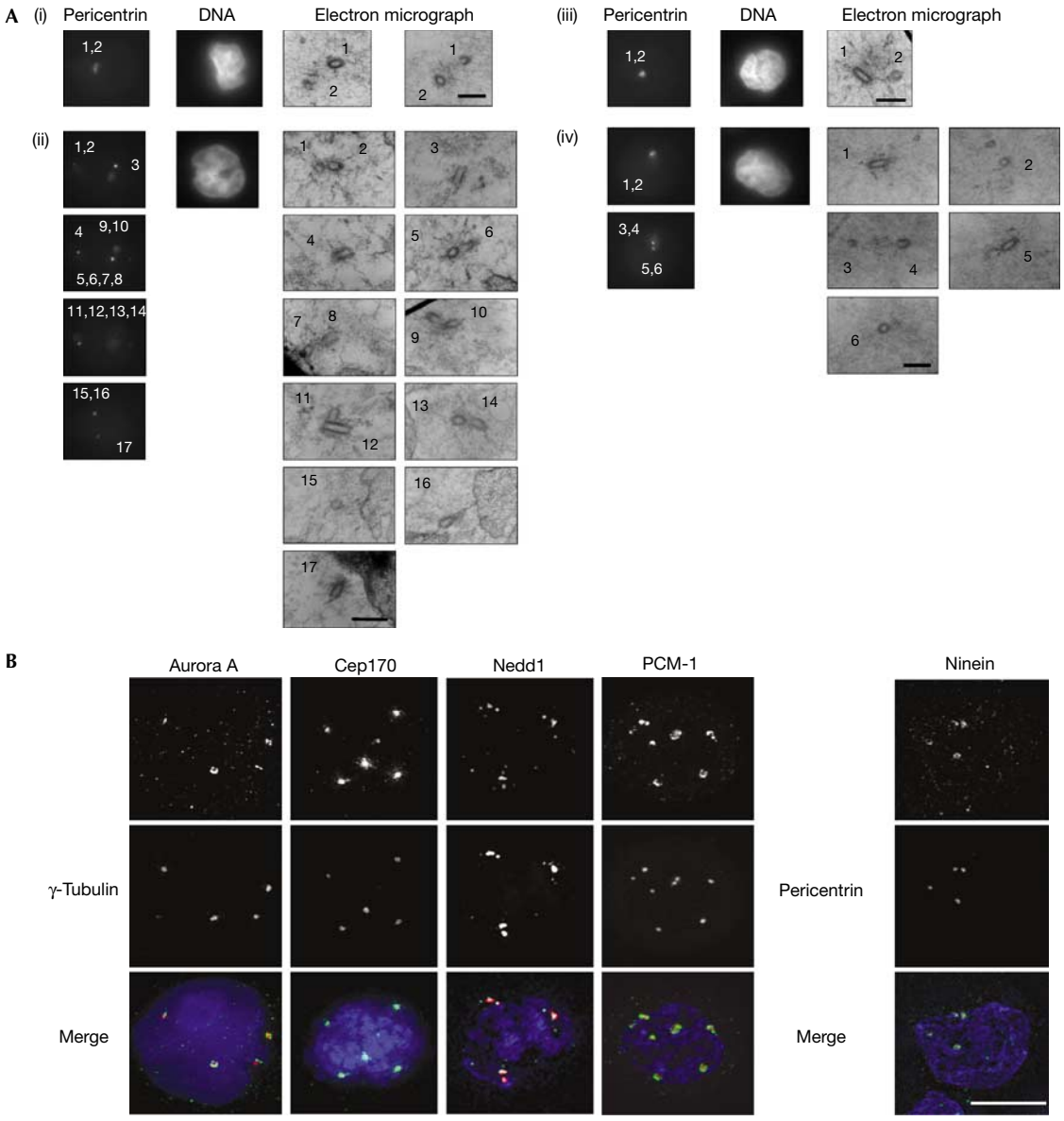

Fig 2 | Microscopy analysis of amplified centrosomes in human cells. (**A**) Electron microscopy was carried out on human control (panels i,ii) or A-T (panels iii,iv) lymphoblastoid cells 48 h after 10 Gy irradiation. Cells were processed for immunofluorescence microscopy of pericentrin and staining of the DNA, as indicated. Cells were then flat-embedded and ultrathin serial sections were cut. Numbers on each pericentrin micrograph indicate the positions of the centrioles shown in the corresponding electron micrographs. Scale bars, 0.5 μm. (**B**) Localization of centrosomal proteins to amplified centrosomes in human lymphoblastoid cells. Cells were stained with antibodies to the indicated centrosomal or pericentrosomal proteins (green) and to γ-tubulin or pericentrin (red), then counterstained with DAPI (blue). Cells shown were fixed and stained 48 h after 10 Gy ionizing radiation treatment. Scale bar, 10 μm. DAPI, 4,6-diamidino-2-phenylindole.

One response that is controlled by complementary activities of ATM and ATR is Chk1 activation. UCN-01 (7-hydroxystaurosporine) is a Chk1 inhibitor (Sarkaria *et al*, 1999; Graves *et al*, 2000). We tested whether disruption of Chk1 function by UCN-01 treatment affected centrosome amplification after ionizing radiation in human lymphoblastoid cells. At the minimum levels of UCN-01 required to inhibit the G2–M checkpoint (Fig 3C), we saw a reduction in the centrosome amplification caused by DNA damage (Fig 3D). At higher UCN-01 concentrations, we noted a complete repression of centrosome amplification (Fig 3D, right panel). Next, we used RNA-mediated interference to knock down the expression of Chk1. Owing to the difficulties we experienced in transfecting the lymphoblastoid cells, we carried out these experiments in U2OS cells. Cells were transfected with inhibitory RNA duplexes and irradiated 24 h after transfection and then analysed 72 h after transfection. As shown in Fig 3E,F, reduction of Chk1 caused a significant repression of centrosome amplification after DNA damage.

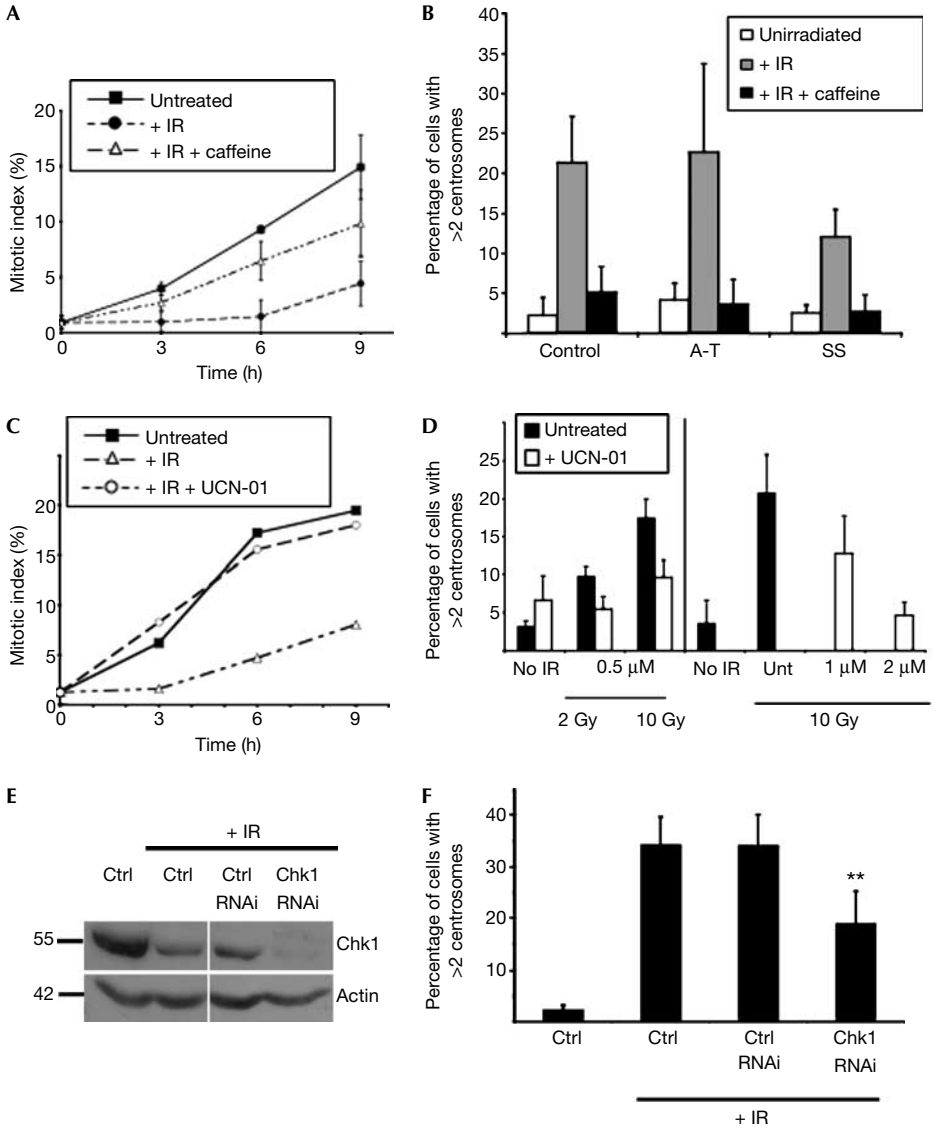

Fig 3 | Dependence of DNA-damage-induced centrosome amplification in human cells on ATM/ATR and Chk1 kinase activity. (**A**) Repression of the G2–M checkpoint by caffeine treatment of human lymphoblastoid cells. Cumulative mitotic indices of human lymphoblastoid cells grown in the presence of colcemid for the time periods indicated after no treatment or after 10 Gy γ-irradiation (IR), with or without preincubation with 2 mM caffeine, as shown. A total of 200 cells were counted per time point in three separate experiments and data presented are the means ± s.d. of these replicates. (**B**) Quantification of control, A-T and Seckel syndrome (SS) cells with multiple centrosomes before and 48 h after 10 Gy ionizing radiation in the presence or absence of caffeine. Centrosomes were counted by immunofluorescence microscopy of γ-tubulin. Data were obtained from at least 500 cells per experiment and histograms show the mean + s.d. of results from three separate blind experiments. (**C**) UCN-01-mediated abrogation of radiation-induced cell-cycle arrest. Cumulative mitotic indices of human lymphoblastoid cells grown in the presence of colcemid for the time points indicated after no treatment or after 2 Gy γ-irradiation, with or without preincubation with 0.5 μM UCN-01. A total of 200 cells were counted per time point. (**D**) UCN-01-mediated suppression of centrosome amplification 48 h after the indicated dose of γ-irradiation in the presence or absence of the indicated concentration of UCN-01. Centrosomes were counted by immunofluorescence microscopy of γ-tubulin. Data were obtained from at least 500 cells per experiment and histograms show the mean + s.d. of results from three separate blind experiments. (**E**) Immunoblot analysis of Chk1 repression in U2OS cells by RNAi at 72 h after transfection, the time at which centrosome counts were performed. Immunoblot for actin was used as a loading control. (**F**) Chk1 RNA interference-mediated suppression of centrosome amplification 48 h after 20 Gy irradiation of U2OS cells. Centrosomes were counted by immunofluorescence microscopy of γ-tubulin. Data were obtained from at least 500 cells per experiment. Asterisks indicate significant difference from irradiated controls (paired *t*-test, $P < 0.01$). ATM, ataxia telangiectasia, mutated; ATR, ATM and Rad3-related; Chk1, checkpoint kinase 1; RNAi, RNA-mediated interference.

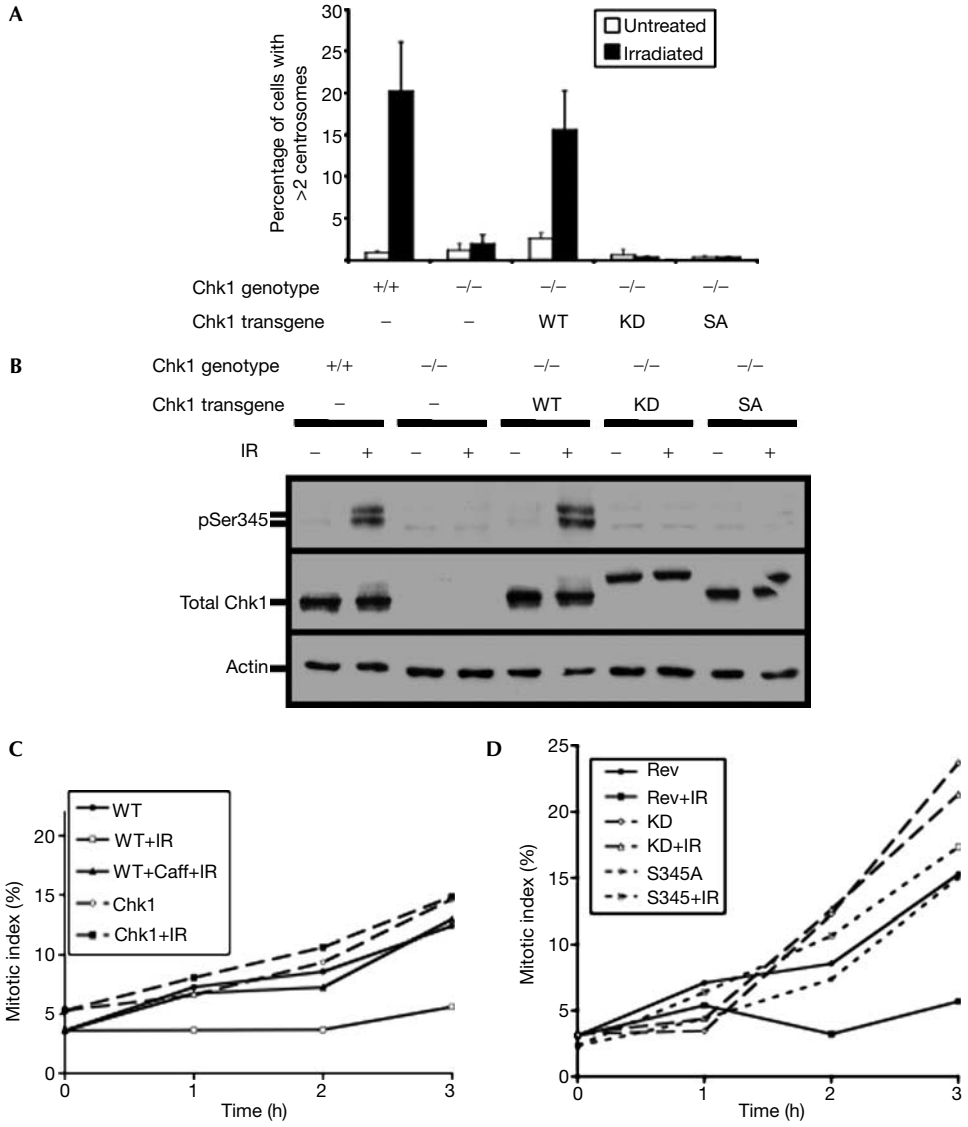

Fig 4 | Genetic analysis of the requirement of Chk1 activity for DNA-damage-induced centrosome amplification. (**A**) Centrosome amplification 12 h after 10 Gy irradiation in *Chk1*$^{-/-}$ DT40 cells and in *Chk1*$^{-/-}$ cells expressing wild-type (WT), kinase-dead (KD) or Ser345Ala (SA) mutant *Chk1* transgenes. Centrosomes were counted by immunofluorescence microscopy of γ-tubulin spots. Data were obtained from at least 500 cells per experiment and histograms show the mean + s.d. of results from three separate blind experiments. (**B**) Immunoblot analysis of Chk1 levels in cells used for the experiments shown in (**A**). Antibodies recognizing Chk1 protein, Chk1 pSer^{345} and actin were hybridized to total cell extract from cells of the indicated genotype before or 2 h after 10 Gy γ-irradiation. (**C**,**D**) Cumulative mitotic indices over time of colcemid-blocked WT, *Chk1*$^{-/-}$*Chk1*$^{+}$ (Rev), *Chk1*$^{-/-}$*Chk1*$^{D130A+}$ (KD) or *Chk1*$^{-/-}$*Chk1*$^{S345A+}$ (SA) cells after no treatment or after 2 Gy γ-irradiation (IR), with or without preincubation with 2 mM caffeine (Caff). A total of 200 cells were counted per time point. Chk1, checkpoint kinase 1.

To confirm the role of Chk1, we turned to the *Chk1*$^{-/-}$ DT40 line (Zachos *et al*, 2003). Chk1-deficient cells showed only background levels of centrosome amplification after irradiation (Fig 4A). When *Chk1* was constitutively expressed in these cells, DNA damage again induced centrosome abnormalities (Fig 4A,B). Notably, the expression of a kinase-dead Chk1 mutant (Asp130Ala) did not restore DNA-damage-induced centrosome amplification (Fig 4A) and also expression of a Chk1 mutant in which serine 345, a PI3K kinase target site, is replaced by alanine (Ser345Ala). Fig 4B confirms the expression of the Chk1 transgenes. The Asp130Ala mutant consistently shows an altered electrophoretic mobility, but repeated sequencing of the expression construct has confirmed that the Asp130Ala alteration is the only mutation. The G2–M checkpoint, which is abrogated by Chk1 deficiency (Zachos *et al*, 2003), was restored by expression of wild-type but not by kinase-dead or Ser345Ala Chk1 (Fig 4C,D).

To test our model of G2-phase centrosome amplification after DNA damage, we carried out fluorescence-activated cell sorting

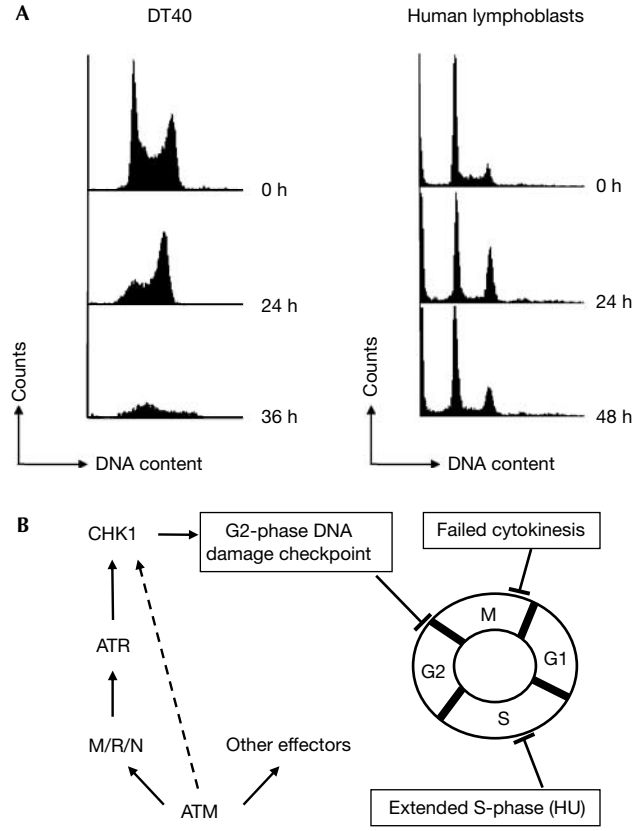

Fig 5 | Cell-cycle analysis after irradiation. (**A**) FACS plots of DT40 and lymphoblast cells at the indicated time points after 10 Gy irradiation. (**B**) Current models for centrosome amplification incorporating the current data on pathways controlling DNA-damage-induced centrosome amplification. FACS, fluorescence-activated cell sorting.

analysis of human and chicken cells after ionizing radiation. We saw no evidence of mitotic failure (polyploid cells) as an alternative explanation for centrosome amplification (Fig 5A). As we also saw Chk1-dependent centrosome amplification occurring after DNA topoisomerase II inhibition (data not shown), we conclude that Chk1-controlled centrosome amplification is a general response to DNA damage in tumour cells and we present the current models (shown in Fig 5B) for how multiple centrosomes might arise. It remains to be defined whether the principal role of Chk1 is the imposition of a cell-cycle delay or whether Chk1 signals directly to the centrosome duplication machinery. Candidate pathways include Cdc25 regulation of Cdk2. The centrosomal localization of Chk1 (Kramer *et al*, 2004) also indicates a potential role at the centrosome.

METHODS

Cloning. Site-directed mutagenesis using a Quikchange kit (Stratagene, La Jolla, CA, USA) was used to replace the sequence encoding Ser 345 or Asp 130 with the sequence for alanine residues in pcDNA3.1zeo-Chk1 (encoding chicken Chk1; Zachos *et al*, 2003) to generate pcDNA3.1zeo-Chk1S345A or pcDNA3.1zeo-Chk1KD, respectively. Constructs were verified by DNA sequencing.

Cell culture and analysis. DT40 cell culture was as described by Dodson *et al* (2004). *Chk1*$^{-/-}$ DT40 cells were transfected with linearized Chk1 expression constructs and overexpressing clones selected as described by Zachos *et al* (2003). Human lymphoblastoid cells GM07521 (apparently normal), GM01525 (A-T) and GM18367 (Seckel syndrome) were obtained from Coriell Cell Repositories and U2OS cells were from the ATCC (Middlesex, UK). UCN-01 was provided by the Division of Cancer Treatment and Diagnosis, National Cancer Institute (Bethesda, MD, USA), and was dissolved in dimethyl sulphoxide at 1 mg/ml. Ionizing radiation experiments were carried out using a ^{137}Cs source at 23.5 Gy/min (Mainance Engineering, Hampshire, UK). Microscopy analysis of mitotic indices and flow cytometry were carried out as described by Dodson *et al* (2004).

RNA-mediated interference. An si*GENOME*^{TM}*SMART* pool of RNA duplexes inhibitory to Chk1 and an si*CONTROL* non-targeting short interfering RNA (siRNA) pool (Dharmacon, Lafayette, CO, USA) were transfected into U2OS cells by using lipofectamine (Invitrogen, Carlsbad, CA, USA). A 300 pmol portion of siRNA was complexed with 12 μl lipofectamine in serum-free medium and added to cells in a 6 ml final volume.

Microscopy. Monoclonal antibodies 3G11 (Dammermann & Merdes, 2002) recognizing pericentrin and 35C1 (Abcam, Cambridge, UK) against Aurora A were used at dilutions of 1:250 and 1:1,000, respectively. Polyclonal antibodies to Cep170 were used at 1:1,000 after methanol fixation (Guarguaglini *et al*, 2005) and antisera to Nedd1, ninein and PCM1 were used as described by Dammermann & Merdes (2002) and Haren *et al* (2006). Immunofluorescence microscopy of pericentrin before electron microscopy and of phospho-H2AX was carried out after fixation with 4% paraformaldehyde and permeabilization with 0.15% Triton X-100, both in cytoskeleton buffer (137 mM NaCl, 5 mM KCl, 1.1 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 2 mM $MgCl_2$, 2 mM EGTA, 5 mM PIPES, 5.5 mM glucose). Further preparation and electron microscopy were carried out as described by Dodson *et al* (2004). Otherwise, immunofluorescence microscopy of pericentrin was carried out as described by Dodson *et al* (2004). Antibodies, cell fixation and staining and light microscopy using an Olympus BX51 microscope were as described previously (Dodson *et al*, 2004).

Immunoblot analysis. Cell extracts were analysed by western blotting as described previously (Zachos *et al*, 2003). We used monoclonal antibodies G4 against Chk1 (Santa Cruz, Santa Cruz, CA, USA) and AC-40 against actin (Sigma-Aldrich, Dublin, Ireland) and polyclonal antibodies against Chk1 phosphorylated at Ser 345 (New England Biolabs, Ipswich, MA, USA) or, for analysis of the RNAi experiment, polyclonal anti-actin (A2066) and monoclonal anti-Chk1 DCS310 (both Sigma).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

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REFERENCES

Balczon R, Bao L, Zimmer WE, Brown K, Zinkowski RP, Brinkley BR (1995) Dissociation of centrosome replication events from cycles of DNA synthesis and mitotic division in hydroxyurea-arrested Chinese hamster ovary cells. *J Cell Biol* **130:** 105–115

Bartek J, Lukas J (2003) Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* **3:** 421–429

Brinkley BR (2001) Managing the centrosome numbers game: from chaos to stability in cancer cell division. *Trends Cell Biol* **11:** 18–21

Dammermann A, Merdes A (2002) Assembly of centrosomal proteins and microtubule organization depends on PCM-1. *J Cell Biol* **59:** 255–266

Dodson H, Bourke E, Jeffers LJ, Vagnarelli P, Sonoda E, Takeda S, Earnshaw WC, Merdes A, Morrison C (2004) Centrosome amplification induced by DNA damage occurs during a prolonged G2 phase and involves ATM. *EMBO J* **23:** 3864–3873

Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF (1996) Abnormal centrosome amplification in the absence of p53. *Science* **271:** 1744–1747

Graves PR, Yu L, Schwarz JK, Gales J, Sausville EA, O'Connor PM, Piwnica-Worms H (2000) The Chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01. *J Biol Chem* **275:** 5600–5605

Griffin CS, Simpson PJ, Wilson CR, Thacker J (2000) Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. *Nat Cell Biol* **2:** 757–761

Guarguaglini G, Duncan PI, Stierhof YD, Holmstrom T, Duensing S, Nigg EA (2005) The forkhead-associated domain protein Cep170 interacts with Polo-like kinase 1 and serves as a marker for mature centrioles. *Mol Biol Cell* **16:** 1095–1107

Guiducci C, Cerone MA, Bacchetti S (2001) Expression of mutant telomerase in immortal telomerase-negative human cells results in cell cycle deregulation, nuclear and chromosomal abnormalities and rapid loss of viability. *Oncogene* **20:** 714–725

Haren L, Remy MH, Bazin I, Callebaut I, Wright M, Merdes A (2006) NEDD1-dependent recruitment of the γ-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. *J Cell Biol* **172:** 505–515

Hut HM, Lemstra W, Blaauw EH, van Cappellen GW, Kampinga HH, Sibon OC (2003) Centrosomes split in the presence of impaired DNA integrity during mitosis. *Mol Biol Cell* **14:** 1993–2004

Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, Jackson SP (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat Cell Biol* **8:** 37–45

Keryer G, Ris H, Borisy GG (1984) Centriole distribution during tripolar mitosis in Chinese hamster ovary cells. *J Cell Biol* **98:** 2222–2229

Kramer A, Mailand N, Lukas C, Syljuasen RG, Wilkinson CJ, Nigg EA, Bartek J, Lukas J (2004) Centrosome-associated Chk1 prevents premature activation of cyclin-B-Cdk1 kinase. *Nat Cell Biol* **6:** 884–891

Lingle WL, Barrett SL, Negron VC, D'Assoro AB, Boeneman K, Liu W, Whitehead CM, Reynolds C, Salisbury JL (2002) Centrosome amplification drives chromosomal instability in breast tumor development. *Proc Natl Acad Sci USA* **99:** 1978–1983

Liu Q *et al* (2000) Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev* **14:** 1448–1459

Meraldi P, Lukas J, Fry AM, Bartek J, Nigg EA (1999) Centrosome duplication in mammalian somatic cells requires E2F and Cdk2-cyclin A. *Nat Cell Biol* **1:** 88–93

Meraldi P, Honda R, Nigg EA (2002) Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in $p53^{-/-}$ cells. *EMBO J* **21:** 483–492

Nigg EA (2002) Centrosome aberrations: cause or consequence of cancer progression? *Nat Rev Cancer* **2:** 815–825

O'Driscoll M, Ruiz-Perez VL, Woods CG, Jeggo PA, Goodship JA (2003) A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. *Nat Genet* **33:** 497–501

Sarkaria JN, Busby EC, Tibbetts RS, Roos P, Taya Y, Karnitz LM, Abraham RT (1999) Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. *Cancer Res* **59:** 4375–4382

Sato C, Kuriyama R, Nishizawa K (1983) Microtubule-organizing centers abnormal in number, structure, and nucleating activity in X-irradiated mammalian cells. *J Cell Biol* **96:** 776–782

Sato N, Mizumoto K, Nakamura M, Tanaka M (2000) Radiation-induced centrosome overduplication and multiple mitotic spindles in human tumor cells. *Exp Cell Res* **255:** 321–326

Shiloh Y (2003) ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* **3:** 155–168

Syljuasen RG, Sorensen CS, Hansen LT, Fugger K, Lundin C, Johansson F, Helleday T, Sehested M, Lukas J, Bartek J (2005) Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol Cell Biol* **25:** 3553–3562

Takai H, Tominaga K, Motoyama N, Minamishima YA, Nagahama H, Tsukiyama T, Ikeda K, Nakayama K, Nakanishi M (2000) Aberrant cell cycle checkpoint function and early embryonic death in $Chk1^{(-/-)}$ mice. *Genes Dev* **14:** 1439–1447

Wong C, Stearns T (2003) Centrosome number is controlled by a centrosome-intrinsic block to reduplication. *Nat Cell Biol* **5:** 539–544

Zachos G, Rainey MD, Gillespie DA (2003) Chk1-deficient tumour cells are viable but exhibit multiple checkpoint and survival defects. *EMBO J* **22:** 713–723

Zachos G, Rainey MD, Gillespie DA (2005) Chk1-dependent S–M checkpoint delay in vertebrate cells is linked to maintenance of viable replication structures. *Mol Cell Biol* **25:** 563–574

Zhao H, Watkins JL, Piwnica-Worms H (2002) Disruption of the checkpoint kinase 1/cell division cycle 25A pathway abrogates ionizing radiation-induced S and G2 checkpoints. *Proc Natl Acad Sci USA* **99:** 14795–14800