Role of *Saccharomyces cerevisiae* Chromatin Assembly Factor-I in Repair of Ultraviolet Radiation Damage *in Vivo*

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ABSTRACT

In vitro, the protein complex Chromatin Assembly Factor-I (CAF-I) from human or yeast cells deposits histones onto DNA templates after replication. In *Saccharomyces cerevisiae*, the *CAC1*, *CAC2*, and *CAC3* genes encode the three CAF-I subunits. Deletion of any of the three *CAC* genes reduces telomeric gene silencing and confers an increase in sensitivity to killing by ultraviolet (UV) radiation. We used double and triple mutants involving *cac1*Δ and yeast repair gene mutations to show that deletion of the *CAC1* gene increases the UV sensitivity of cells mutant in genes from each of the known DNA repair epistasis groups. For example, double mutants involving *cac1*Δ and excision repair gene deletions *rad1*Δ or *rad14*Δ showed increased UV sensitivity, as did double mutants involving *cac1*Δ and deletions of members of the *RAD51* recombinational repair group. *cac1*Δ also increased the UV sensitivity of strains with defects in either the error-prone (*rev3*Δ) or error-free (*pol30-46*) branches of *RAD6*-mediated postreplicative DNA repair but did not substantially increase the sensitivity of strains carrying null mutations in the *RAD6* or *RAD18* genes. Deletion of *CAC1* also increased the UV sensitivity and rate of UV-induced mutagenesis in *rad5*Δ mutants, as has been observed for mutants defective in error-free postreplicative repair. Together, these data suggest that CAF-I has a role in error-free postreplicative damage repair and may also have an auxiliary role in other repair mechanisms. Like the *CAC* genes, *RAD6* is also required for gene silencing at telomeres. We find an increased loss of telomeric gene silencing in *rad6*Δ *cac1*Δ and *rad18*Δ *cac1*Δ double mutants, suggesting that CAF-I and multiple factors in the postreplicative repair pathway influence chromosome structure.

IN *Saccharomyces cerevisiae*, the genes *CAC1*, *CAC2*, and *CAC3* encode the three subunits of Chromatin Assembly Factor-I (CAF-1). These genes encode proteins homologous to human CAF-I subunits (Kaufman *et al.* 1997). *In vitro*, CAF-I from several organisms deposits histones onto DNA templates that have undergone replication or nucleotide excision repair synthesis (Smith and Stillman 1989; Gaillard *et al.* 1996; Kamakaka *et al.* 1996; Kaufman *et al.* 1997). Also, deletion of any of the three yeast *CAC* loci confers a modest increase in sensitivity to killing by ultraviolet (UV) radiation, implying a DNA repair defect. Combining the three *cac* gene deletions in the same strain does not give rise to further UV sensitivity, suggesting that the CAF-I protein complex becomes nonfunctional with respect to DNA repair upon loss of any one subunit (Kaufman *et al.* 1997).

We wished to explore the nature of the repair defect conferred by *cac* gene deletions. Most biochemical analysis of DNA repair has been performed using naked DNA *in vitro* (*e.g.*, Aboussekhra *et al.* 1995). However, DNA repair in cells occurs in the context of chromatin proteins, and reformation of nucleosomes after repair is also required. Previous biochemical data have shown that CAF-I can catalyze nucleosome formation during nucleotide excision repair *in vitro* (Gaillard *et al.* 1996). However, it is not clear how these *in vitro* data relate to the *in vivo* UV-sensitive phenotype. For example, CAF-I could have a role in excision repair, but other proteins might be able to substitute for CAF-I during this process *in vivo*, so that the observed UV sensitivity of *cac* mutants might be unrelated to excision repair. *S. cerevisiae* provides an opportunity to determine which repair pathways are defective in *cac* mutants, by double mutant analysis using null alleles of previously characterized repair genes.

In yeast, genes that influence cellular sensitivity to killing by ultraviolet radiation fall into three major groups that are usually considered to control three different types of DNA repair (Game and Cox 1973; Cox and Game 1974). These repair mechanisms function in the dark and are distinct from light-mediated photoreactivation, which represents a fourth type of repair (Harm 1976). The different groups of repair genes were initially defined by genetic analysis (Game and Cox 1973). Double mutant strains carrying mutations in two genes within the same group show a UV sensitivity no greater than that of one of the component single mu-

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tants, whereas double mutant combinations involving mutations in different groups display increased sensitivity. The three epistasis groups defined in this way are referred to by a prominent locus in each group, and they mediate repair by different mechanisms. Thus, the *RAD1* group mediates nucleotide excision repair, an error-free mechanism by which thymine dimers and other UV photoproducts are recognized and excised from the DNA, after which DNA synthesis and ligation restores the DNA duplex (reviewed by Sancar 1994). The *RAD51* group mediates repair by recombinational mechanisms (reviewed by Game 1993). This is the major repair mechanism for DNA double-strand breaks in yeast, and mutants in the *RAD51* group are highly sensitive to ionizing radiation. In addition, they are mildly sensitive to UV radiation, because base damage also triggers recombinational processes. The *RAD6* group mediates postreplicative repair: in wild-type cells, newly replicated low-molecular-weight DNA strands are rapidly converted to intact high-molecular-weight DNA in the presence of polymerase-blocking UV photoproducts. In contrast, *rad6* mutants are unable to perform this method of repair, and gaps in DNA result when DNA polymerases are blocked by UV-induced photoproducts (di Caprio and Cox 1981; Prakash 1981; reviewed in Prakash *et al.* 1993 and Lawrence 1994). The *RAD6* group is involved in both error-free and error-prone repair of these gaps; thus, *rad6* mutants are highly UV sensitive and display greatly reduced levels of UV-induced mutagenesis (Lawrence and Christensen 1976). *rad6* mutants are also defective in various aspects of chromosome function, as discussed below.

To determine which of the different repair pathways is affected in the *cac* mutants, we constructed double mutant strains incorporating a *cac1*Δ deletion and a mutation in a member of each epistasis group and determined the UV sensitivity of these strains. We also studied some triple mutant strains. We observed that deletion of the *CAC1* gene increased the UV sensitivity of at least some mutants from each of the three epistasis groups. In contrast, there was almost no increase in sensitivity in *rad6*Δ *cac1*Δ and *rad18*Δ *cac1*Δ double mutants.Therefore, we propose that CAF-I functions to assist multiple *RAD6*-mediated repair reactions.

Like CAF-I, Rad6p participates in the formation of telomeric chromatin structures that mediate epigenetic silencing of telomere-proximal genes (Enomoto *et al.* 1997; Huang *et al.* 1997; Kaufman *et al.* 1997). However, mutants lacking *RAD18*, another gene in the *RAD6* epistasis group, display wild-type levels of telomeric silencing (Huang *et al.* 1997). Here, we show that deletion of both *CAC1* and either *RAD6* or *RAD18* causes more severe telomeric silencing defects than those observed in the single mutants. These data suggest that CAF-I and the Rad6p/Rad18p proteins contribute independently to chromatin structures responsible for telomeric gene silencing.

MATERIALS AND METHODS

Yeast strains and plasmids: Genotypes of relevant strains are given in Table 1. All strains used were isogenic, derived from strain W303 (Thomas and Rothstein 1989) by transformation or by crossing with other strains in this background. We note that the W303 strain background carries a point mutation in the *RAD5* gene, known as the *rad5-535* allele (Fan *et al.* 1996). However, this mutation causes very little UV sensitivity, far less than a *rad5*Δ deletion allele (Fan *et al.* 1996), and we observed that *rad5*Δ *cac1*Δ strains display significantly greater UV sensitivity and UV-induced mutation rates than *rad5-535 cac1*Δ strains (Figures 4 and 6). Together, these data suggest that the *rad5-535* allele functions at a nearly wild-type level.

The *cac1*Δ*::LEU2* and *URA3-VIIL* alleles were described previously (Kaufman *et al.* 1997). To construct the *cac1*Δ*::hisG-URA3-hisG* allele, plasmid pPK98 carrying a 5.6-kb genomic *Bam*HI fragment with the *CAC1* gene (Kaufman *et al.* 1997) was first digested with *Bgl*II and *Nhe*I. A 5.4-kb *Bam*HI-*Xba*I fragment containing the *URA3* gene and a kanamycin-resistance gene flanked by a direct repeat of bacterial DNA was inserted into the digested pPK98 to generate pPK102. pPK102 was digested with *Bam*HI and used to transform wild-type diploid W303-1 cells to Ura^+ prototrophy. Correct integration was confirmed by Southern blotting. Mutant alleles in W303-based strains provided by others were as follows: strain W1214-19b (*rad1*Δ*::LEU2*) and strain U687 (*rad51*Δ*::LEU2*) from R. Rothstein; strain HKY578-2C (*rad5*Δ*::URA3*) from Hannah Klein (Fan *et al.* 1996); *rad14*Δ*::LEU2* from Richard Verhage; strain BL31-2c (*rad52*Δ*::TRP1*) from A. Lustig (Li and Lustig 1996); and strains DLY67 (*MAT***a** *rad53* (*mec2-1*)) and DLY262 (*MAT*α *rad9*Δ*::HIS3 rad24*Δ*::TRP1*) from Ted Weinert. Plasmids used to make disruptions were pYPG101 (*rev3*Δ*::hisG-URA3-hisG*), gift of C. Lawrence; pBL243 (*pol30*Δ*::hisG-URA3-hisG*) (Ayyagari *et al.* 1995), gift of P. Burgers; and pJJ239 (*rad18*Δ*::hisG-URA3-hisG*) and pR671 (*rad6*Δ*::hisG-URA3-hisG*), gifts of L. Prakash, as were plasmids pR67 (*RAD6*), pR661 ($rad6_{\Delta 1\text{-}9}$), and pSCW231 (Watkins *et al.* 1993).

Disruptions were made by lithium acetate transformation of a wild-type diploid as described by Kaiser *et al.* (1994). Selected colonies were colony-purified twice after transformation and then sporulated. The *pol30*Δ deletion was confirmed by Southern blotting; the lethality of this deletion was complemented by plasmids carrying the *POL30* gene (see Figure 4C). For *RAD6*, *RAD18*, and *REV3* gene deletions, the radiation-sensitive phenotype cosegregated with the expected nutritional marker in the progeny tetrads. We confirmed that the correct gene had been disrupted in each case by demonstrating inability of the new deletion alleles to complement known alleles of the appropriate *RAD* or *REV* gene. Loss of the *URA3* gene in the *cac1*Δ, *rad6*Δ, and *rad18*Δ disruption strains was obtained by counterselection using 5-fluoro-orotic acid (FOA) as described by Alani *et al.* (1987).

Genetic procedures and media: Standard procedures for genetic crosses and tetrad analysis were used. Standard yeast media for crosses and for scoring genetic marker segregations were those of Kaiser *et al.* (1994). YPAD is YPD medium supplemented with 50 mg/liter of adenine. FOA was added to synthetic complete medium at a concentration of 1 mg/ml.

Ultraviolet radiation source and survival curves: Logarithmically growing cells ($\sim 10^7$ cells/ml) were assessed for UV sensitivity by preparing a dilution series in distilled water and plating immediately on solid YEPD medium or on the appropriate selective media (−Trp) to maintain the plasmids for the experiments in Figure 4A. Each plate was then irradiated for an appropriate time using a shielded apparatus containing six General Electric G8T5 tubes giving most of their radiation at

TABLE 1

Yeast strains used

Strain number	Reference	Genotype
PKY027	Kaufman *et al.* (1997)	*MAT*α
PKY028	Kaufman *et al.* (1997)	*MAT***a**
PKY020	Kaufman *et al.* (1997)	MAT**a** *cac1*Δ*::LEU2*
PKY048	This work	*MAT***a** *rad9*Δ*::HIS3*
PKY051	This work	*MAT***a** *rad9*Δ*::HIS3 cac1*Δ*::LEU2*
PKY065	This work	*MAT***a** *rad52*Δ*::TRP1*
PKY067	This work	*MAT***a** *rad52*Δ*::TRP1 cac1*Δ*::LEU2*
DLY67	T. Weinert	*MAT***a** *rad53(mec2-1)*
PKY070	This work	*MAT***a** *rad53(mec2-1) cac1*Δ*::LEU2*
g1052-2b	This work	*MAT***a** *rad1*Δ*::LEU2*
g1052-2d	This work	*MAT*α *rad1*Δ*::LEU2 cac1*Δ*::hisG-URA3-hisG*
g1062-1C	This work	*rad14*Δ*::LEU2*
g1062-1A	This work	*rad14*Δ*::LEU2 cac1*Δ*::hisG-URA3-hisG*
HKY578-2C	Fan *et al.* (1996)	MAT**a** *rad5*Δ*::URA3*
PKY099	This work	*MAT***a** *rad5*Δ*::URA3 cac1*Δ*::LEU2*
g1057-1a	This work	*MAT***a** *rad6*Δ*::hisG-URA3-hisG*
g1061-12b	This work	*MAT*α *rad6*Δ*::hisG-URA3-hisG cac1*Δ*::LEU2*
g1060-4a	This work	*MAT*α *rad18*Δ*::hisG-URA3-hisG*
g1060-4b	This work	*MAT*α *rad18*Δ*::hisG-URA3-hisG cac1*Δ*::LEU2*
g1054-1b	This work	*MAT*α *rad51*Δ*::LEU2*
g1054-7d	This work	*MAT*α *rad51*Δ*::LEU2 cac1*Δ*::hisG-URA3-hisG*
g1053-4b	This work	*MAT***a** *rad1*Δ*::LEU2 rad52*Δ*::TRP1*
g1053-10b	This work	*MAT*α *rad1*Δ*::LEU2 rad52*Δ*::TRP1 cac1*Δ*::hisG-URA3-hisG*
g1058-3b	This work	*MAT***a** *rad6*Δ*::hisG-URA3-hisG rad52*Δ*::TRP1*
g1061-1b	This work	*MAT*α *rad6*Δ*::hisG-URA3-hisG rad52*Δ*::TRP1 cac1*Δ*::LEU2*
PKY090	Kaufman *et al.* (1997)	*MAT***a** *URA3-VIIL*
PKY106	Kaufman *et al.* (1997)	*MAT***a** *URA3-VIIL cac1*Δ*::LEU2*
PKY487	This work	*MAT*α *rad18*Δ*::hisG*
PKY488	This work	*MAT*α *rad18*Δ*::hisG cac1*Δ*::LEU2*
PKY489	This work	*MAT*α *rad6*Δ*::hisG*
PKY490	This work	*MAT***a** *rad6*Δ*::hisG cac1*Δ*::LEU2*
PKY508	This work	*MAT***a** *URA3-VIIL rad6*Δ*::hisG*
PKY506	This work	*MAT***a** *URA3-VIIL rad6*Δ*::hisG cac1*Δ*::LEU2*
PKY507	This work	*MAT***a** *URA3-VIIL rad18*Δ*::hisG*
PKY505	This work	*MAT***a** *URA3-VIIL rad18*Δ*::hisG cac1*Δ*::LEU2*
PKY656	This work	*MAT***a** *rad6*Δ*::hisG* + pR661 (*rad6*$_{\Delta 1-9}$)
PKY659	This work	*MAT***a** *rad6*Δ*::hisG cac1*Δ*::LEU2* + pR661 (*rad6*$_{\Delta 1-9}$)
PKY718	This work	*MAT***a** *rev3*Δ*::hisG-URA3-hisG*
PKY721	This work	*MAT***a** *rev3*Δ*::hisG-URA3-hisG cac1*Δ*::LEU2*
PKY741	This work	*MAT***a** *pol30*Δ*::hisG-URA3-hisG* + pBL230 (*POL30*)
PKY743	This work	*MAT***a** *pol30*Δ*::hisG-URA3-hisG* + pBL230-46 (*pol30-46*)
PKY766	This work	*MAT***a** *pol30*Δ*::hisG-URA3-hisG cac1*Δ*::LEU2* + pBL230 (*POL30*)
PKY768	This work	*MAT***a** *pol30*Δ*::hisG-URA3-hisG cac1*Δ*::LEU2* + pBL230-46 (*pol30-46*)

All strains were in the background W303 and carried the following genetic markers in addition to the ones listed above: *leu2-3, 112 ura3-1 his3-11,15 trp1-1 ade2-1 can1-100*.

254 nm. Plates were incubated in the dark for 4 days and survival was assessed by counting visible colonies. Survival curves were performed several times with consistent results, and single curves representative of the results are shown in the figures. Error bars on the survival curves represent twice the standard error based on the number of colonies counted for the point in question for the chosen curve. In most cases these bars are smaller than the symbol used to delimit the point. This method assumes a Poisson distribution for the number of cells in random samples of equal volume for a given suspension (Scheffler 1979).

Mutagenesis experiments: Logarithmically growing cells were harvested at a density of ~6×10^6/ml, washed with distilled water, and plated immediately in duplicate on solid YPAD medium to count viable cell number (~200 surviving cells/plate at each UV dose), and on synthetic media lacking either adenine or tryptophan (~$1–2 \times 10^7$ total cells/plate) to measure *ade2-1* or *trp1-1* reversion. After UV irradiation at the indicated doses, plates were incubated in the dark at 30° and survival was assessed by counting visible colonies on YPAD plates after 3 days. Ade^+ and Trp^+ revertants were counted after 6 days.

Telomeric silencing assays: To measure telomeric silencing, the *URA3-VIIL*-marked telomere was used (Kaufman *et al.* 1997; originally described in Gottschling *et al.* 1990). This assay monitors silencing of a wild-type *URA3* gene that is in-

serted near a telomere. Silenced cells exhibit a Ura$^-$ phenotype, which includes resistance to FOA, whereas nonsilenced cells are Ura$^+$ and FOA sensitive. We found that equivalent results were obtained by using log phase cells ($A_{600} = \sim 0.6$) from liquid medium or with cells taken from plates and adjusted to $A_{600} = \sim 0.6$ (*e.g.*, Rundlett *et al.* 1996). To assess the frequency of silenced cells in a population, cells were plated on synthetic complete + FOA medium, and the number of FOA-resistant colonies per cell plated was determined after 8 days of incubation at 30°. Small FOA-resistant microcolonies formed by the *rad18*Δ *cac1*Δ *URA3-VIIL* strain were counted under a dissecting microscope, and the 8-day incubation was necessary to fully detect these microcolonies. Colonies on synthetic complete medium plates were counted after 3 days to assess the number of viable plated cells. In order to correct for variation in the potency of the FOA in different batches of plates, the fraction of FOA-resistant cells was normalized to that of the wild-type strain for each repetition of the experiment.

RESULTS

Epistasis analysis of the *cac1*Δ UV-sensitivity phenotype: Previous work has shown that deletion of any of the three *CAC* loci confers an increase in sensitivity to UV and that double and triple *cac*Δ mutant combinations confer no additional sensitivity (Kaufman *et al.* 1997). We sought to determine whether the UV sensitivity of a *cac*Δ mutant was related to a defect in DNA damage repair mediated by one of the known epistasis groups of *rad* mutants. We therefore crossed strains carrying a *cac1*Δ deletion to isogenic strains with representative *rad*Δ deletions and measured the UV sensitivity of the resulting double mutants to determine which type of DNA repair is affected upon loss of CAF-I.

To test the relationship between *CAC1* and recombinational repair, we first obtained UV survival data for single and double mutants involving *CAC1* and each of two genes in this epistasis group, *RAD51* and *RAD52* (Figure 1). As previously reported (Kaufman *et al.* 1997), the *cac1*Δ allele conferred moderate UV sensitivity, which was less severe than that conferred by the *rad51*Δ and *rad52*Δ mutations. These in turn were less sensitive than mutants in the other UV epistasis groups (*e.g.*, Cox and Game 1974; Figures 1–3), because recombination is more important for repair of double-strand breaks than for repairing UV photoproducts. We observed an increase in sensitivity in double mutants involving *cac1*Δ and either *rad51*Δ (Figure 1A) or *rad52*Δ (Figure 1B).

Several genes involved in sensing DNA damage also confer a radiation-sensitive phenotype when mutated, including the *RAD9* and *RAD53* genes (Weinert and Hartwell 1988; Allen *et al.* 1994; Weinert *et al.* 1994). To determine whether UV sensitivity of *cac1* mutants is related to this function, we obtained UV survival data for *cac1*Δ in combination with a *rad9*Δ deletion and an allele of the *RAD53* gene. We used a missense allele of *RAD53* previously described as *mec2-1* (Weinert *et al.* 1994), because *RAD53* is essential for viability. The *rad9*Δ *cac1*Δ double mutant (Figure 1C) and *rad53 cac1*Δ double mutant (Figure 1D) were both more UV sensitive than the *rad CAC1*$^+$ strains.

To test the relationship between *CAC1* and nucleotide excision repair, the UV sensitivity of double mutants involving the excision repair-defective mutations *rad1*Δ and *rad14*Δ in combination with *cac1*Δ were compared with the *rad*Δ single mutants (Figure 2). The Rad1p protein directly binds the Rad10p protein, forming an endonuclease complex that performs an incision step during nucleotide excision repair (Bardwell *et al.* 1994). *RAD14* encodes a protein homologous to the human xeroderma pigmentosum group A (XPA) protein (Bankmann *et al.* 1992); like XPA, Rad14p recognizes DNA containing UV photoproducts (Guzder *et al.* 1993). The *rad1*Δ *cac1*Δ double mutant displayed increased UV sensitivity compared to the *rad1*Δ mutant strain (Figure 2A). For *rad14*Δ combined with *cac1*Δ (Figure 2B), there was an equivalent or slightly larger increase in sensitivity.

To confirm the assignment of *CAC1* outside of the *RAD1* and *RAD52* epistasis groups for UV repair using a more sensitized assay, we constructed a triple mutant strain lacking *CAC1*, *RAD1*, and *RAD52*. Blocking two repair pathways will direct more UV-induced damage into the remaining pathway, thus increasing the apparent sensitivity caused by mutations in the remaining pathway (Game and Cox 1973). Thus, we predicted that the increased sensitivity between *rad1*Δ and *cac1*Δ would be demonstrated more readily when these mutants are also deleted for *RAD52*. Comparison of survival data for a triple mutant strain with data for a *rad1*Δ *rad52*Δ double mutant showed that a further increase in sensitivity was in fact conferred by the *cac1*Δ mutation (Figure 2C).

To test the relationship between *CAC1* and postreplicative DNA repair, we measured the UV sensitivity caused by combination of the *cac1*Δ mutation with deletions of *RAD6* epistasis group members (Figure 3). *rad6*Δ and *rad18*Δ are the two most UV-sensitive mutants defective in postreplicative DNA repair, blocked for both error-free and error-prone mechanisms (reviewed in Prakash *et al.* 1993; Lawrence 1994). The *cac1*Δ mutation did not substantially increase the UV sensitivity of a *rad6*Δ (Figure 3A) or a *rad18*Δ strain (Figure 3B), even at doses at which *cac1*Δ did increase the UV sensitivity of *rad1*Δ and *rad14*Δ mutants (Figure 2). A triple mutant combination involving *cac1*Δ with both *rad6*Δ and *rad52*Δ also showed only a very small increase in UV sensitivity (Figure 3C), in contrast to the situation for the *rad1*Δ *rad52*Δ *cac1*Δ triple mutant (Figure 2C). Together these data suggest that most of the UV resistance conferred by CAF-I action requires the Rad6p protein (see discussion).

Because the *rad6*Δ and *rad18*Δ deletions block the activity of multiple proteins involved in distinct error-free and error-prone repair mechanisms, we sought to determine whether CAF-I function could be assigned

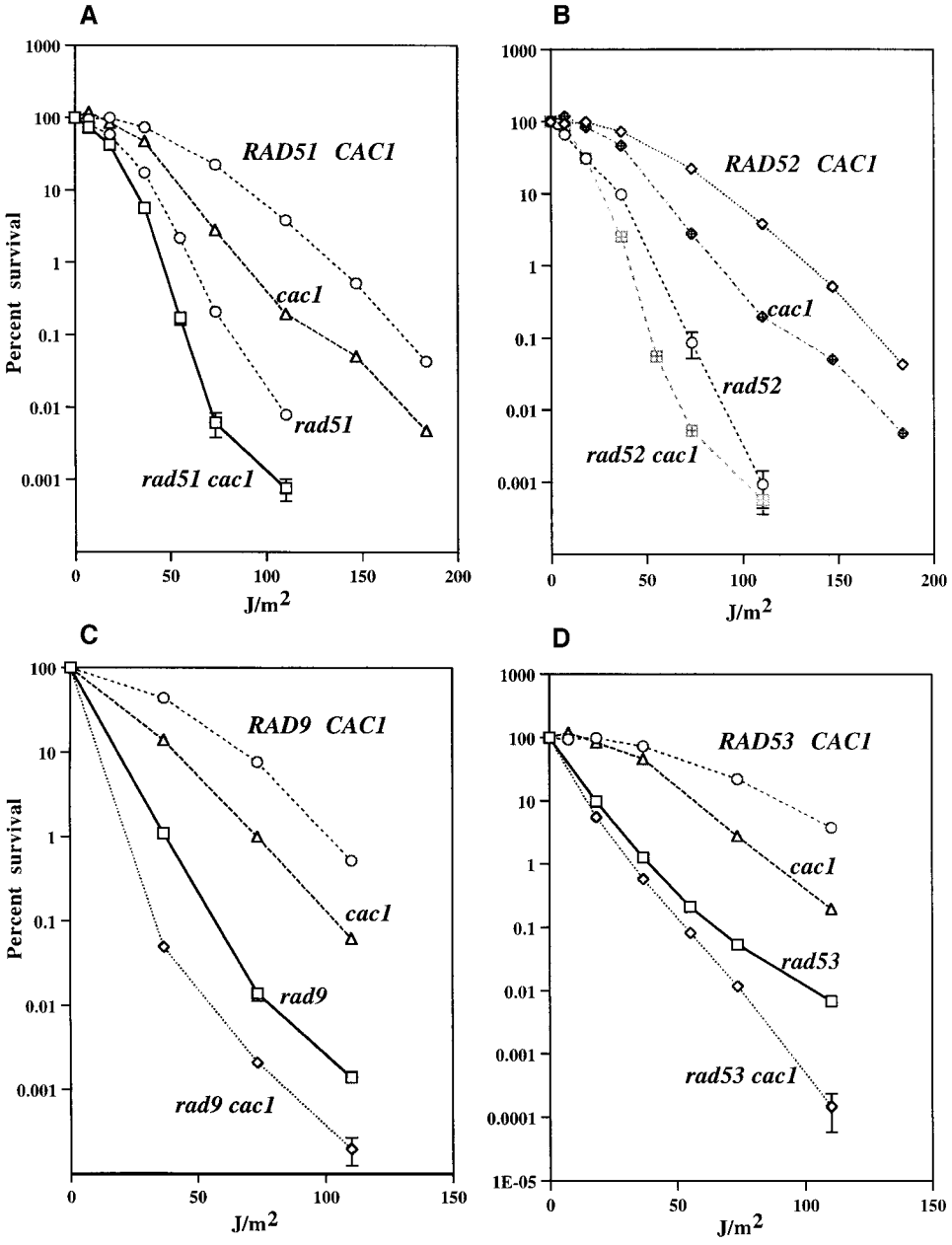

Figure 1.—UV survival data for wild-type, single mutant, and double mutant strains involving *cac1*Δ and *rad*Δ mutations in the recombinational repair group and mutations in cell cycle checkpoint genes. (A) *cac1*Δ in combination with *rad51*Δ. (B) *cac1*Δ in combination with *rad52*Δ. (C) *cac1*Δ in combination with *rad9*Δ. (D) *cac1*Δ in combination with *rad53* (*mec2-1* allele). Strains used were as follows: PKY028, *RAD CAC1*; PKY020, *cac1*Δ; g1054-1B, *rad51*Δ; g1054-7D, *cac1*Δ *rad51*Δ; PKY065, *rad52*Δ; PKY067, *rad52*Δ *cac1*Δ; PKY048, *rad9*Δ; PKY051, *cac1*Δ *rad9*Δ; DLY67, *rad53-(mec2-1)*; and PKY070, *cac1*Δ *rad53-(mec2-1)*.

to any particular subset of the *RAD6* epistasis group. We therefore tested a less UV-sensitive *RAD6* allele termed $rad6_{\Delta 1\text{-}9}$, which encodes a protein lacking the highly conserved N-terminal nine residues. $rad6_{\Delta 1\text{-}9}$ cells display increased UV-induced mutagenesis rates with respect to wild type, contrary to *rad6*Δ mutants, which display almost no UV-induced mutagenesis (Lawrence and Christensen 1976; Watkins *et al.* 1993). This suggests that deletion of the Rad6p N terminus results primarily in a defect in error-free postreplicative repair (Watkins *et al.* 1993). Combination of *cac1*Δ with $rad6_{\Delta 1\text{-}9}$ caused a substantial increase in UV sensitivity (Figure 4A).

We also tested *cac1*Δ in combination with mutations in other genes that affect error-free subsets of the *RAD6*-mediated repair processes. For example, *rad5*Δ mutants are much less UV sensitive than *rad6*Δ or *rad18*Δ mutants, and deletion of *RAD5* does not increase the UV sensitivity of *rad6*Δ or *rad18*Δ mutants (Johnson *et al.* 1992). *RAD5* contributes to error-free postreplicative repair: deletion of *RAD5* increases the UV sensitivity of *rev3*Δ strains that lack the error-prone polymerase zeta (Johnson *et al.* 1992), and *rad5*Δ strains display increased rates of nonhomologous recombination reactions (Ahne *et al.* 1997). As observed for the $rad6_{\Delta 1\text{-}9}$ mutant, a *rad5*Δ *cac1*Δ double mutant strain was more UV sensitive than a *rad5*Δ strain (Figures 4B and 6). To further test the relationship between CAF-I and error-free postreplicative DNA repair, we also tested the effects of *cac1*Δ on *pol30-46* strains. The *pol30-46* allele contains four separate point mutations, each of which changes a charged residue to alanine in the gene encoding PCNA (Proliferating Cell Nuclear Antigen), which

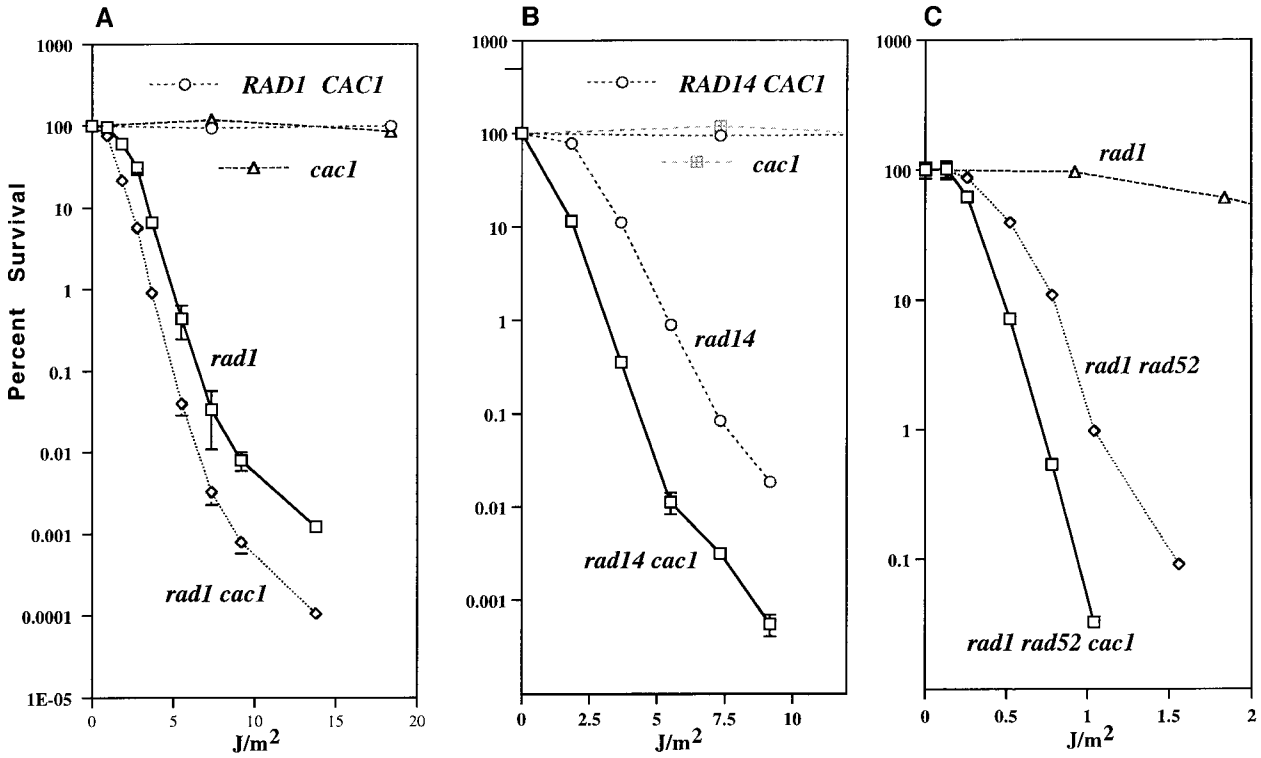

Figure 2.—UV survival data for wild-type and mutant strains involving *cac1*Δ and *rad*Δ mutations in the excision repair group. (A) *cac1*Δ in combination with *rad1*Δ. (B) *cac1*Δ in combination with *rad14*Δ. (C) *cac1*Δ in a triple mutant combination with *rad1*Δ and *rad52*Δ, compared to a *rad1*Δ *rad52*Δ double mutant. Strains used were as follows: PKY028, *RAD CAC1*; PKY020, *cac1*Δ; g1052-2B, *rad1*Δ; g1052-2D, *cac1*Δ *rad1*Δ; g1062-1C, *rad14*Δ; g1062-1A, *rad14*Δ *cac1*Δ; g1053-4b, *rad1*Δ *rad52*Δ; and g1053-10b, *rad1*Δ *rad52*Δ *cac1*Δ.

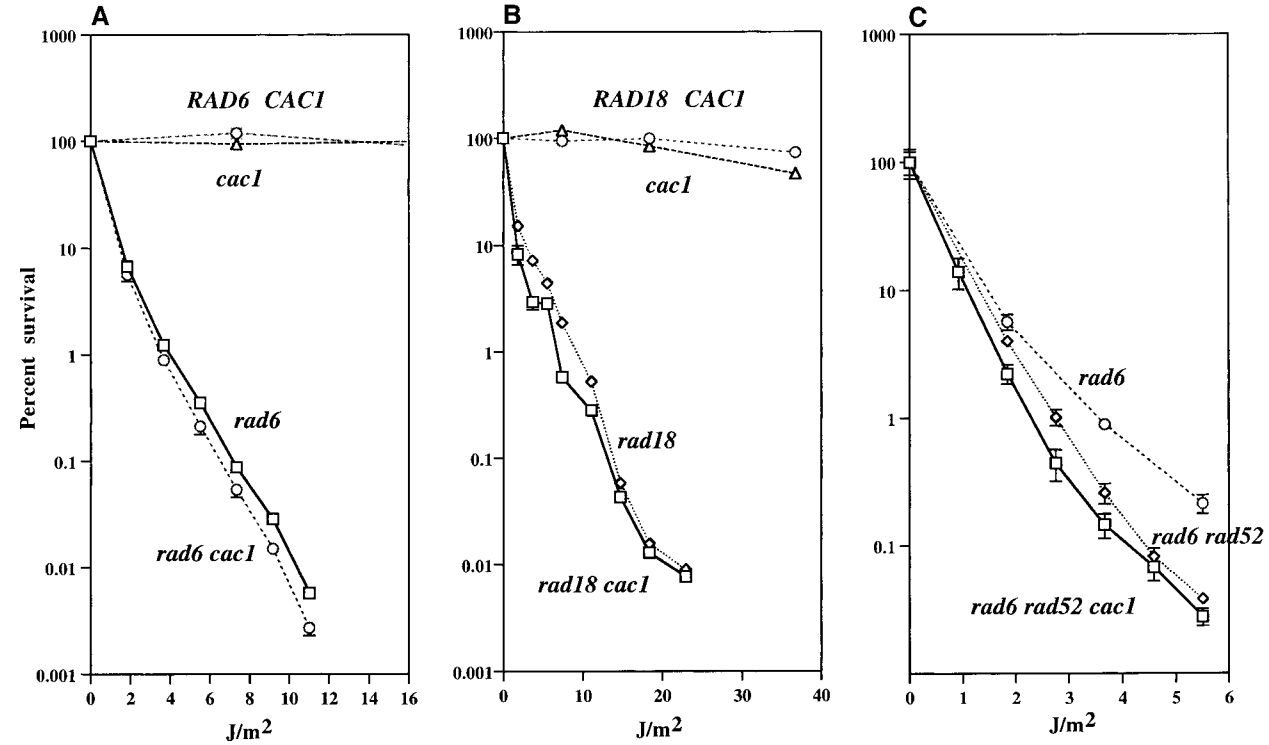

Figure 3.—UV survival data for wild-type and mutant strains involving *cac1*Δ and deletions of *RAD6* or *RAD18*. (A) *cac1*Δ in combination with *rad6*Δ. (B) *cac1*Δ in combination with *rad18*Δ. (C) *cac1*Δ in a triple mutant combination with *rad6*Δ and *rad52*Δ, compared to a *rad6*Δ *rad52*Δ double mutant. Strains used were as follows: PKY028, *RAD CAC1*; PKY020, *cac1*Δ; g1057-1A, *rad6*Δ; g1060-12B, *rad6*Δ *cac1*Δ; g1060-4A, *rad18*Δ; g1060-4B, *rad18*Δ *cac1*Δ; g1058-3b, *rad6*Δ *rad52*Δ; and g1061-1b, *rad6*Δ *rad52*Δ *cac1*Δ.

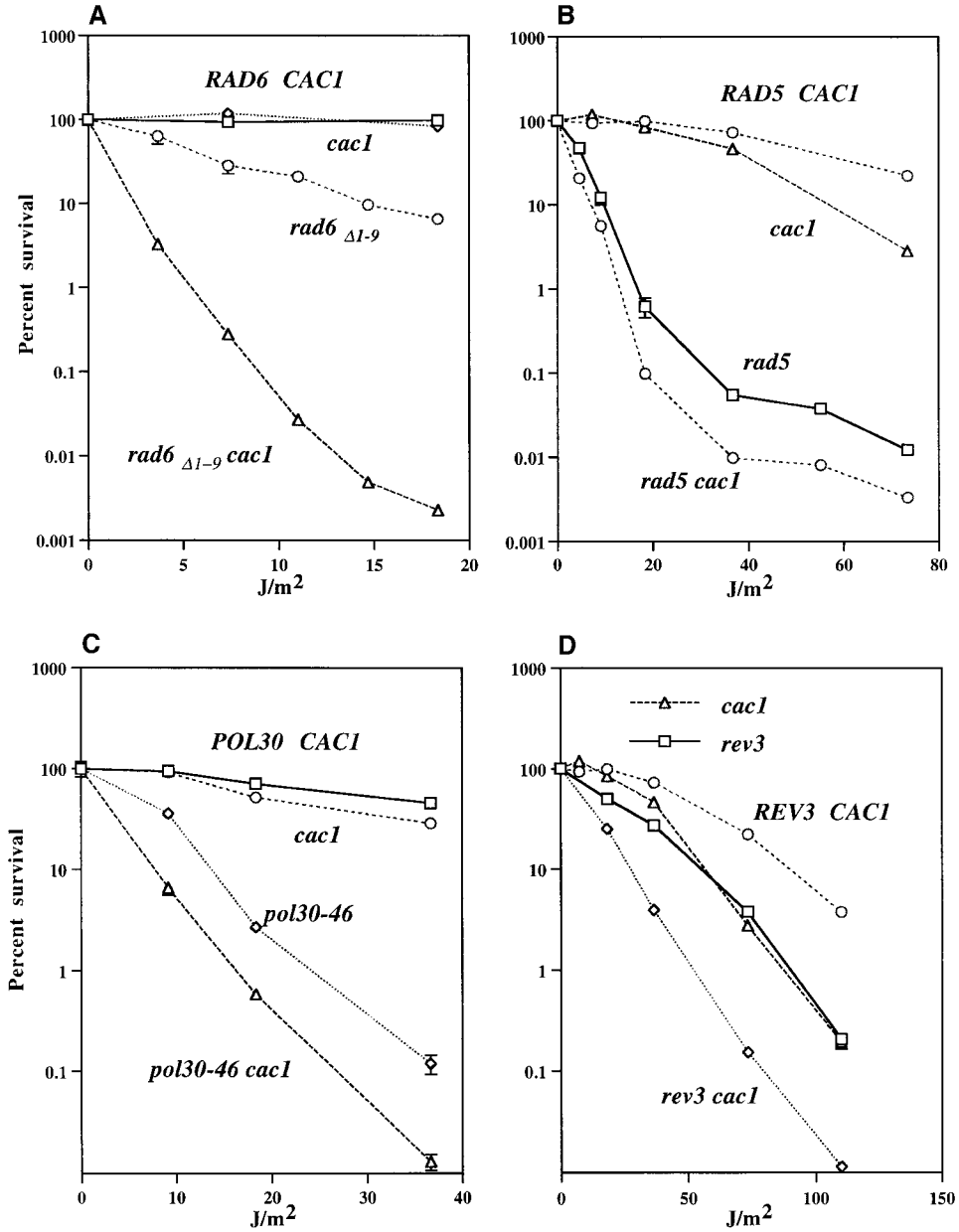

Figure 4.—UV survival data for wild-type and mutant strains involving *cac1*Δ and mutations affecting subsets of the *RAD6*/*RAD18*-mediated repair pathways. (A) *cac1*Δ in combination with $rad6_{\Delta 1\text{-}9}$. (B) *cac1*Δ in combination with *rad5*Δ. (C) *cac1*Δ in combination with *pol30-46*. (D) *cac1*Δ in combination with *rev3*Δ. Strains used were as follows: PKY028, *RAD CAC1*; PKY020, *cac1*Δ; PKY656, *rad6*Δ + pR661 ($rad6_{\Delta 1\text{-}9}$); PKY659, *rad6*Δ *cac1*Δ + pR661 ($rad6_{\Delta 1\text{-}9}$); HKY578-2C, *rad5*Δ; PKY099, *rad5*Δ *cac1*Δ; PKY741, *pol30*Δ *CAC1* + pBL230 (*POL30*); PKY743, *pol30*Δ *CAC1* + pBL230-46 (*pol30-46*); PKY766, *pol30*Δ *cac1*Δ + pBL230 (*POL30*); PKY768, *pol30*Δ *cac1*Δ + pBL230-46 (*pol30-46*); PKY718, *rev3*Δ; PKY721, *cac1*Δ *rev3*Δ.

is required for the processivity of eukaryotic leading-strand polymerase delta (Prelich *et al.* 1987; Tsurimoto and Stillman 1989). *pol30-46* encodes a PCNA protein able to support viability and able to stimulate polymerases *in vitro*, but this allele appears to block most, if not all, error-free postreplicative repair, because *pol30-46 rev3*Δ strains, which also lack *RAD6*-mediated error-prone repair, are nearly as UV sensitive as *rad6*Δ strains (Torres-Ramos *et al.* 1996). We observed that a *pol30-46 cac1*Δ strain was more UV sensitive than a *pol30-46* strain (Figure 4C). In summary, the *cac1*Δ deletion increased the UV sensitivity of three mutants, *rad5*Δ, $rad6_{\Delta 1\text{-}9}$, and *pol30-46*, known to be defective in error-free postreplicative repair. This suggests that CAF-I may be required for more than one subset of *RAD6*-mediated repair mechanisms, or controls a subset distinct from that affected by the mutations tested (see discussion).

We also tested the effects of *cac1*Δ on a mutant defective in error-prone repair. *rev3* mutants lack the error-prone DNA polymerase zeta, are mildly UV sensitive, and lack UV-induced mutagenesis (Lawrence and Christensen 1976; Nelson *et al.* 1996). We observed that a *rev3*Δ *cac1*Δ double mutant strain was more UV sensitive than a *rev3*Δ strain (Figure 4D).

Levels of UV-induced mutagenesis in a *cac1*Δ mutant: Thymine dimers and other photoproducts generated by UV light result in gapped DNA following replication (di Caprio and Cox 1981; Prakash 1981). Wild-type cells are able to fill these gaps by both error-free and error-prone mechanisms. Both these mechanisms are defective in *rad6* and *rad18* mutants (di Caprio and

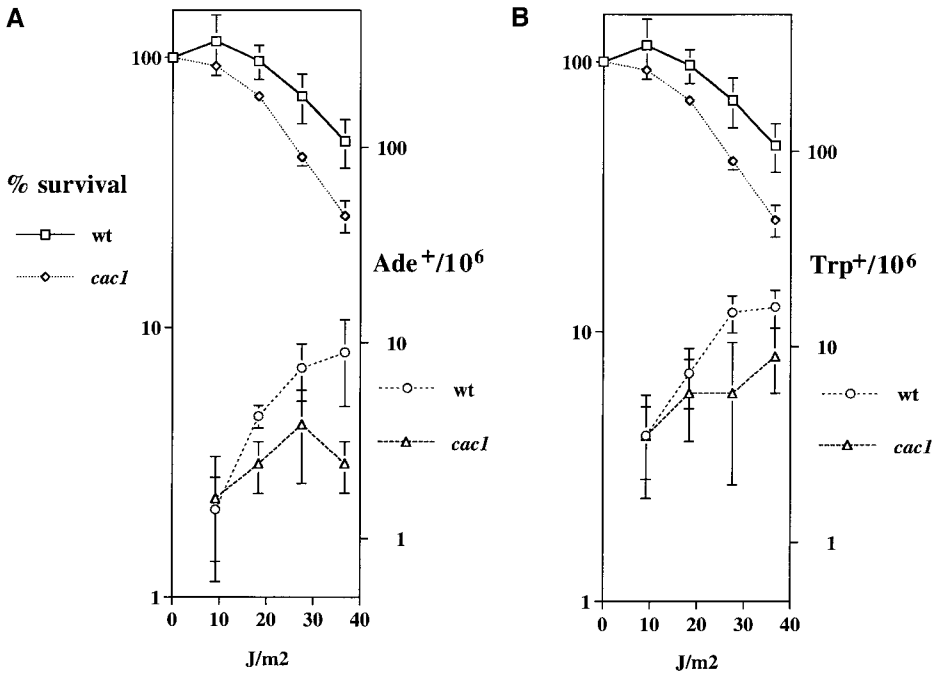

Figure 5.—UV-induced mutagenesis in wt and *cac1*Δ strains. Strains used were PKY090 (*MAT***a**, URA3-VIIL) and isogenic derivative PKY106 (*cac1*Δ*::LEU2*). Survival of the strains at the UV doses used is plotted in each graph on the *y* axis on the left. (A) Ade^+ revertants per 10^6 viable cells is plotted *vs.* the UV doses given. (B) Trp^+ revertants per 10^6 viable cells is plotted *vs.* the UV doses given. Each experimental point was repeated at least three times; average values with standard deviations (bars) are shown.

Cox 1981; Prakash 1981); therefore, these mutants display reduced levels of UV-induced mutagenesis (Lawrence and Christensen 1976; Cassier-Chauvat and Fabre 1991; Armstrong *et al.* 1994). In contrast, nucleotide excision repair mutants inefficiently remove the photoproducts that stimulate postreplicative repair, including error-prone repair mechanisms, causing increased UV-induced mutagenesis levels (see, *e.g.*, Lawrence and Christensen 1976). If CAF-I were involved in nucleotide excision repair *in vivo*, *cac1*Δ mutants would be expected to display at least a slightly increased rate of UV-induced mutagenesis compared to wild type.

We therefore examined UV-induced mutagenesis in a *cac1*Δ mutant. Reversion of two point mutations (*ade2-1* and *trp1-1*) present in the W303-1 strain background was measured for several UV doses in wild-type and *cac1*Δ cells (Figure 5). At both loci, no increase in induced mutagenesis frequencies was observed for the *cac1*Δ mutant compared to wild type.

Mutation of genes in the error-free subset of the *RAD6* group in some cases increases levels of UV-induced mutagenesis (Watkins *et al.* 1993; Broomfield *et al.* 1998). This presumably occurs because damage normally repaired by nonmutagenic mechanisms is instead repaired by the mutagenic DNA polymerase zeta encoded by the *REV3* and *REV7* genes. Such increases in mutagenesis can occur as the result of mutation of more than one gene: loss of both the *RAD5* and *RAD30* genes leads to a large synergistic increase in UV-induced mutagenesis (McDonald *et al.* 1997). We observed that *cac1*Δ significantly increased UV mutability in *cac1*Δ *rad5*Δ double mutants compared to *rad5*Δ single mutants (Figure 6). This suggests that CAF-I does play a role in error-free postreplicative DNA repair (see discussion).

Telomeric gene silencing in *cac1*Δ, *rad6*Δ, and *rad18*Δ mutants: In wild-type cells, transcription of a *URA3* gene adjacent to telomeres is largely suppressed by telomeric gene silencing. This results in a fraction of cells in a population being resistant to the drug FOA (Gottschling *et al.* 1990), which is rendered toxic by activity of the *URA3* gene (Boeke *et al.* 1987; see also materials and methods). In these experiments, *cac* mutant strains with a *URA3*-marked telomere inserted next to the left telomere of chromosome VII generated an average of fivefold fewer FOA-resistant cells than wild type (Table 2), indicative of a reduced level of telomeric *URA3* silencing. As observed previously, the colonies that did grow on FOA were smaller than those from a wild-type strain, suggesting that the ability to silence *URA3* transcription is transient in *cac* mutants (data not shown, but see Enomoto *et al.* 1997; Kaufman *et al.* 1997; Monson *et al.* 1997). *rad6*Δ mutants also displayed reduced levels of telomeric gene silencing (Huang *et al.* 1997); in our strain background, the frequency of FOA-resistant colonies was ~20-fold less than wild type, and the colonies were also smaller than in wild type (Table 2 and data not shown). As previously observed, *rad18*Δ mutants displayed approximately wild-type levels of telomeric silencing (Table 2; Huang *et al.* 1997; see below).

Because our data suggested that *CAC1* may function partially outside of the *RAD6* epistasis group with respect to UV sensitivity (Figure 4), we asked whether there was also an independent contribution of these genes to telomeric gene silencing. Indeed, double mutant combinations of *cac1*Δ and either *rad6*Δ or *rad18*Δ had more severe defects in telomeric silencing than the single mutants (Table 2). We observed that *rad6*Δ *cac1*Δ double mutants produced FOA-resistant colonies at a frequency ~10^{-4} that of a wild-type strain; *cac1*Δ and *rad6*Δ single mutant strains generated average FOA-resistant

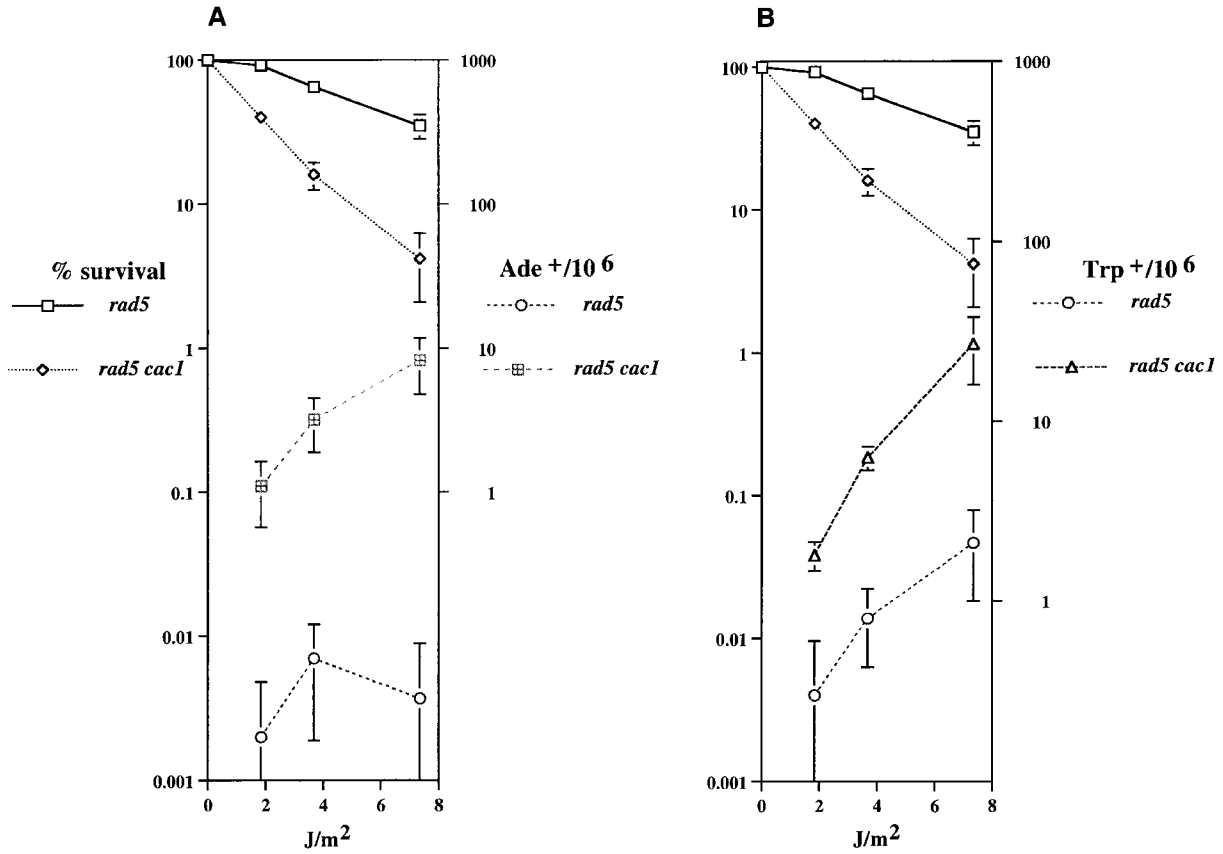

Figure 6.—UV-induced mutagenesis in *rad5*Δ and *rad5*Δ *cac1*Δ strains. Strains used were HKY578-2C (*rad5*Δ) and isogenic derivative PKY099 (*rad5*Δ *cac1*Δ). Survival of the strains at the indicated UV doses (left axes). (A) Ade$^+$ or (B) Trp$^+$ revertants per 10^6 viable cells is plotted *vs.* the UV doses (right axes). Each experimental point was repeated three times; average values with standard deviations (bars) are shown.

frequencies of 0.2 and 0.05 that of wild type, respectively. The reduction of telomeric silencing was also dramatic in the case of the *rad18*Δ *cac1*Δ double mutant, which generated full-size FOA-resistant colonies at an average frequency of 2×10^{-4} that of wild type. In addition, as observed for *cac* mutants, the *rad18*Δ *cac1*Δ strain generated FOA-resistant microcolonies, but in this case, the microcolonies were far smaller than those observed for *cac* mutants, and required the use of a dissecting microscope in order to count them. The microscopic FOA-resistant *rad18*Δ *cac1*Δ colonies arose at an average frequency of 2×10^{-2} that of the full-size wild-type colonies. Even considering microcolonies, this resistance value is an order of magnitude below the level of FOA-resistance observed in the same experiments for the *cac1*Δ mutant alone. Control experiments using strains lacking the *URA3-VIIL* marker showed that none of the mutations tested caused cells to become intrinsically sensitive to FOA (Table 2). Thus, the observed effects result from changes in telomeric gene silencing.

DISCUSSION

Contribution of *CAC1* to UV repair: *S. cerevisiae* cells lacking any of the *CAC1*, *2*, or *3* genes encoding the three subunits of CAF-I display increased UV sensitivity (Kaufman *et al.* 1997). We show here that loss of the *CAC1* gene increased the UV sensitivity of *rad51*Δ and *rad52*Δ mutants (Figure 1). These data indicate that *CAC1* does not belong to the *RAD51* epistasis group and is therefore unlikely to be involved in recombinational repair. This conclusion is consistent with previous data showing that cells lacking any or all of the three *CAC* genes display no increase in sensitivity to gamma-irradiation (Kaufman *et al.* 1997), a treatment that causes double-strand breaks that are normally repaired by the recombination pathway (reviewed by Game 1993). *cac1*Δ also increased the sensitivity of *rad9*Δ and *rad53 (mec2-1)* mutants, suggesting that *CAC1* does not protect cells from UV damage through a role in S-phase checkpoint control (Figure 1). Similarly, *cac1*Δ increased the sensitivity of *rad1*Δ, *rad14*Δ, and *rad1*Δ *rad52*Δ mutants (Figure 2). These data place *CAC1* outside of the *RAD1* epistasis group responsible for nucleotide excision repair.

*cac1*Δ mutant cells display no increase in UV-induced mutagenesis compared to wild-type cells (Figure 5). Increased mutagenesis is a common phenotype of mutants defective in nucleotide excision repair mediated by the *RAD1* epistasis group (see, *e.g.*, Lawrence and Chris-

TABLE 2

Telomeric gene silencing in *cac1*Δ, *rad6*Δ, and *rad18*Δ mutant combinations

Strain	Relevant genotype	Relative fraction FOA-resistant	*n*
PKY090	*URA3-VIIL*	1.0	6
PKY106	*URA3-VIIL cac1*Δ	0.20 ± 0.13	6
PKY505	*URA3-VIIL rad6*Δ	$(5.2 \times 10^{-2}) \pm (5.6 \times 10^{-2})$	6
PKY506	*URA3-VIIL rad6*Δ *cac1*Δ	$(9.5 \times 10^{-5}) \pm (5.8 \times 10^{-5})$	6
PKY507	*URA3-VIIL rad18*Δ	2.9 ± 1.9	5
PKY508	*URA3-VIIL rad18*Δ *cac1*Δ	$(1.9 \times 10^{-4}) \pm (1.8 \times 10^{-4})$ large colonies	5
PKY508	*URA3-VIIL rad18*Δ *cac1*Δ	$(2.0 \times 10^{-2}) \pm (2.4 \times 10^{-2})$ microscopic colonies	4
PKY028	wt	0.63	2
PKY020	*cac1*Δ	0.77	2
PKY487	*rad18*Δ	0.92	2
PKY488	*rad18*Δ *cac1*Δ	0.75	2
PKY489	*rad6*Δ	0.73	2
PKY490	*rad6*Δ *cac1*Δ	0.86	2

The fraction of FOA-resistant cells in populations of the indicated strains was determined relative to growth on synthetic complete media. The values were normalized to a value of 1.0 for the wild-type strain for each independent experiment, and the adverage ± the standard deviation for multiple experiments (*n*) was determined. For the *URA3-VIIL rad18*Δ *cac1*Δ strain, the microscopic FOA-resistant colonies observed were counted separately from the colonies visible to the eye.

To test whether the gene deletions examined caused an intrinsic sensitivity to FOA, the fraction of FOA-resistant colonies in strains lacking the *URA3-VIIL* telomere was measured. The unnormalized average value obtained in two experiments is reported. All FOA-resistant colonies were the same size (*i.e.*, not microscopic) for each strain lacking the *URA3*-marked telomere. Therefore, decreased levels of FOA resistance in the strains containing the *URA-VIIL* telomere result from reductions in gene silencing.

tensen 1976). Therefore, this result is consistent with the UV-sensitivity data placing *CAC1* outside the *RAD1* excision repair group. However, CAF-I from vertebrate cells does deposit histones onto DNA templates undergoing nucleotide-excision repair *in vitro* (Gaillard *et al.* 1996), reflecting the ability of CAF-I to recognize (directly or indirectly) DNA polymerase movement in a cell-free system. We hypothesize that other factors may be more important than or functionally redundant with CAF-I *in vivo* for reformation of nucleosomes after nucleotide-excision repair. Alternatively, poor nucleosome reformation after nucleotide-excision repair may not impact viability. Because both nucleotide excision repair and error-free postreplicative repair involve polymerases stimulated by PCNA (Ayyagari *et al.* 1995; Torres-Ramos *et al.* 1996), one possibility suggested by these data is that these proteins are recognized by CAF-I as the cue to specifically target DNA replicated during repair for nucleosome assembly and that this targeting has lower specificity *in vitro*.

No significant increase in UV sensitivity was detected when *cac1*Δ was combined with *rad6*Δ or *rad18*Δ alleles (Figure 3). We note that at similarly low UV doses (5–10 J/m^2), deletion of *CAC1* was observed to significantly increase the UV sensitivity of *rad1*Δ and *rad14*Δ mutants (Figure 2). Furthermore, deletion of *CAC1* had little effect on the UV sensitivity of a *rad6*Δ *rad52*Δ double mutant (Figure 3C). Because *rad6*Δ *rad52*Δ cells are able to survive UV damage almost exclusively by action of the remaining *RAD1*-dependent nucleotide excision repair pathway, this supports our conclusion that nucleotide excision repair is largely functional in *cac1*Δ mutants.

What aspect of *RAD6*-mediated postreplicative repair is affected by loss of CAF-I? Error-prone repair appears to be intact in *cac1*Δ mutants: *cac1*Δ increased the UV sensitivity of nonmutagenic *rev3*Δ strains (Figure 4), and UV-induced mutagenesis is at near-wild-type levels in *cac1*Δ cells (Figure 5). The strong increase in UV sensitivity observed when *cac1*Δ was combined with *rad5*Δ, *pol30-46*, or $rad6_{\Delta 1\text{-}9}$ mutations implies that CAF-I also operates outside of many known *RAD6*-dependent error-free repair functions (Figure 4). However, the increase in UV-induced mutagenesis in *rad5*Δ *cac1*Δ strains compared to *rad5*Δ single mutants (Figure 6) suggests a role for CAF-I in error-free postreplicative repair analogous to that observed for the *RAD30* gene (McDonald *et al.* 1997). In that case, deletion of *RAD5* in a *rad30*Δ strain also increased UV-induced mutagenesis, presumably because mutation of multiple error-free repair factors leads to increased damage repair by the error-prone polymerase zeta. Overall, we interpret our data to suggest that the majority of the UV-protective effect of CAF-I action occurs via Rad6p- and Rad18p-mediated error-free mechanisms.

Our data are consistent with two models for how

CAF-I contributes to DNA repair. In one scenario, CAF-I acts as an auxiliary factor to assist in DNA repair by multiple pathways. For example, if CAF-I were important for multiple subsets of *RAD6*-mediated DNA repair, then it would be expected that *cac1*Δ deletions would increase the UV sensitivity of all mutants tested except *ra6*Δ and *rad18*Δ deletions, as observed. A second possibility is that nucleosome assembly by CAF-I results in some prevention of ultraviolet radiation damage to the DNA, and that in the absence of CAF-I the amount of damage caused by a given UV dose increases. This would result in the observed increase in UV sensitivity caused by *cac* gene deletions in combination with almost any *rad* mutation (Figures 1–4). We note that *cac1* mutants have enlarged nuclei (Enomoto *et al.* 1997); perhaps this phenotype directly or indirectly causes the DNA to become more easily damaged upon UV irradiation. We note that these two possibilities are not mutually exclusive.

***RAD6* and *CAC1* both affect chromosome function:** *RAD6* and *CAC1* each contribute to position-dependent gene silencing in yeast (Enomoto *et al.* 1997; Huang *et al.* 1997; Kaufman *et al.* 1997; Monson *et al.* 1997; Enomoto and Berman 1998; Kaufman *et al.* 1998). In addition, the *RAD6* gene is required for several other biological processes including postreplicative DNA repair, UV-induced mutagenesis and N-end-dependent protein degradation (Dohmen *et al.* 1991; reviewed in Prakash *et al.* 1993; Lawrence 1994). Rad6p is a member of the E2 family of ubiquitin-conjugating enzymes (Jentsch *et al.* 1987); mutation of the catalytic cysteine required for formation of ubiquitin conjugates destroys the biological activity of the protein with respect to all known phenotypes (Sung *et al.* 1990, 1991; Huang *et al.* 1997). Other genes in the *RAD6* group with regard to UV sensitivity are generally involved in only a subset of these processes and often have quantitatively less severe phenotypes than *rad6* mutants (reviewed in Prakash *et al.* 1993; Lawrence 1994).

rad18 mutants are highly UV sensitive (Figure 3; Lawrence and Christensen 1976) and are also defective in postreplicative repair (Prakash 1981). Rad18p is a single-strand DNA-binding protein that directly interacts with Rad6p to form a heterodimer (Bailly *et al.* 1994, 1997a,b). Mutation of the Rad6p-binding domain of Rad18p leads to a UV-sensitivity phenotype very similar to that of deletion alleles (Bailly *et al.* 1997a). Such data suggest that Rad18p recruits Rad6p to single-stranded DNA segments that remain after DNA synthesis through damaged regions. The strong UV-sensitive phenotype of *rad18*Δ mutants suggests that this interaction is critical for the ability of Rad6p to function in the recovery from DNA damage.

Several phenotypes related to chromosome structure have been observed in *rad6* mutants. Ty transposition is stimulated and displays a more randomized insertion pattern in *rad6* mutants (Picologlou *et al.* 1990; Liebman and Newnam 1993), and deletion of *RAD6* reduces the transcriptional silencing and increases mitotic recombination of Ty elements located in ribosomal RNA genes (Bryk *et al.* 1997). In contrast, recombination within the *ARS1* locus is reduced in a *rad6* mutant (Markvart *et al.* 1996). Also, there are effects on heterochromatic gene silencing in *rad6* mutants, including diminished gene silencing at telomeres and the *HML* silent mating-type locus (Huang *et al.* 1997; Figure 7). The catalytic cysteine of Rad6p is required for this function, suggesting that ubiquitination of an unknown substrate is required for silencing. Consistent with this hypothesis, a Sir-protein-binding deubiquitinating enzyme acts to antagonize telomeric silencing (Moazed and Johnson 1996). Although Rad6p is able to ubiquitinate histones *in vitro* (Sung *et al.* 1988), it is not certain that these are the biologically relevant substrates *in vivo* (Swerdlow *et al.* 1990). *RAD6* is also required for successful meiosis in yeast (Game *et al.* 1980). Likewise, disruption of a *RAD6*-homologous gene in mice causes male sterility arising from defects in spermatogenesis during postmeiotic chromatin remodeling, suggesting that aspects of *RAD6* function have been conserved in evolution (Roest *et al.* 1996). Together, these data suggest that global aspects of chromosome structure are perturbed in the absence of *RAD6* function.

We note that *rad18*Δ mutants display no defects in telomeric silencing (Huang *et al.* 1997), but that loss of *RAD6* or *RAD18* further reduces telomeric silencing in the absence of *CAC1* (Table 2). This suggests that that Rad6p and CAF-I play nonredundant roles in the formation of the proper chromatin structure for telomeric gene silencing. One possibility for the role of Rad18p is that it is normally functionally redundant with other factors that serve to recruit Rad6p to telomeric DNA, but that in the absence of CAF-I, these other factor(s) are unable to function properly, presumably due to changes in chromatin structure. This may result in a much more substantial reduction in Rad6p recruitment to telomeres in the absence of both CAF-I and Rad18p, leading to the observed synergistic silencing defect.

Although Rad18p appears to be functionally redundant with other factors with respect to telomeric silencing, this is clearly not the case for DNA repair. The strong UV-sensitive phenotype of *rad18*Δ mutants instead suggests that Rad18p is the most important factor for recruitment of Rad6p to sites of DNA damage, and cannot be substituted in this role regardless of the presence of CAF-I.

Recent results (Singh *et al.* 1998) demonstrate that in the fission yeast *Schizosaccharomyces pombe*, *rhp6* mutants defective in the gene structurally and functionally homologous to *S. cerevisiae RAD6* also have position-dependent gene silencing defects at their silent mating loci. These silencing defects display a novel specificity: the silent mating cassettes are only derepressed in *rhp6* mutant cells when the *cis*-acting sequences allowing for

mating type switching are intact. This implies that the recombinational switching event itself causes a requirement for *rhp6*$^{+}$ function with regard to silencing; the authors propose a model in which proper reassembly of chromatin after the switch is the critically regulated event.

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