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The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle

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DNA double-strand breaks (DSBs) are dangerous lesions that can lead to genomic instability and cell death. Eukaryotic cells repair DSBs either by nonhomologous end-joining (NHEJ) or by homologous recombination. We investigated the ability of yeast cells (*Saccharomyces cerevisiae*) to repair a single, chromosomal DSB by recombination at different stages of the cell cycle. We show that cells arrested at the G_1 phase of the cell cycle restrict homologous recombination, but are able to repair the DSB by NHEJ. Furthermore, we demonstrate that recombination ability does not require duplicated chromatids or passage through S phase, and is controlled at the resection step by Clb–CDK activity.

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Introduction

DNA can be damaged as a result of failures during replication or exposure to damage-inducing agents at any stage of the cell cycle. One of the most genotoxic lesions is a DNA double-strand break (DSB). Nonhomologous end-joining (NHEJ) and homologous recombination are the principal pathways responsible for repairing DSBs in eukaryotes. Defects in either repair pathway result in high frequencies of genomic instability (Ferguson *et al*, 2000; Kolodner *et al*, 2002), underscoring the importance of these processes as caretakers of the genome. NHEJ is a mechanism able to join DNA ends with no, or minimal, homology, whereas repair of DSBs by recombination requires a homologous sequence as a template. Choice between these genetically and enzymatically distinct repair pathways should be tightly regulated, but cellular variables determining pathway commitment have remained elusive. In addition, to ensure survival, DSB repair should be coordinated within the cell cycle. Progression within the eukaryotic cell cycle is primarily determined by the sequential action of cyclin-dependent kinases (CDKs). A single CDK, Cdc28, controls cell cycle in budding yeast. Association of Cdc28 with one of three G_1 cyclins (*CLN*s) is necessary for entering the cell cycle (START) (Tyers *et al*, 1993). The Cln–CDK complex, in turn, promotes the transcription of the S and G_2 cyclins (*CLB*s), responsible for the activation of a number of pathways, including DNA replication, bud emergence and spindle pole body duplication (Nasmyth, 1993). Premature entry into the S phase is precluded by high levels of Sic1, a stoichiometric inhibitor of Clb–CDK activity (Mendenhall, 1993). Upon destruction of Sic1 by the APC/cyclosome, Clb–CDK activity increases, shutting down Cln–CDK activity, thus triggering DNA replication (Lengronne and Schwob, 2002).

Here, we have investigated the ability of yeast cells to repair a single, chromosomal DSB at different stages of the cell cycle. We show that DSBs created at the G_1 stage of the cell cycle are not repaired by homologous recombination. Instead, the broken chromosomes are repaired by NHEJ. Our analysis shows that homologous repair is not dependent on DNA replication, or the presence of duplicated chromatids. Rather, Clb–CDK activity is the essential feature required for recombinational repair of DSBs. Furthermore, we show that Clb–CDK activity is required to carry out resection, one of the earliest stages of the homologous recombination process.

Results and discussion

We have designed haploid strains of the yeast *Saccharomyces cerevisiae* that bear two copies of the *URA3* gene; one of them, located on chromosome V, carries the recognition site for the yeast HO site-specific endonuclease (*ura3-HOcs*). The second copy, located ectopically on chromosome II, carries a single-base pair mutation that prevents HO recognition (*ura3-HOcs-inc*), flanked on either side by two restriction sites, *Bam*HI and *Eco*RI. In these strains, the HO gene is under the transcriptional control of a galactose-inducible promoter. Upon transfer to galactose-containing medium, the enzyme creates a single DSB in each cell of the population. The broken chromosomes are then repaired by homologous recombination, during which the *HOcs-inc* and the flanking markers are copied, resulting in a gene conversion event (Figure 1A). During the repair, the donor chromosome remains unchanged and there is no loss of viability. There is no need for genetic selection of recombination products; instead, repair is monitored in the entire cell population (Aylon and Kupiec, 2003; Aylon *et al*, 2003).

In these strains, ectopic donor sequences are present throughout the cell cycle, thus enabling us to investigate homologous repair capability at different cell cycle stages. We arrested cells at G_1 (with alpha-factor) or G_2 (with nocodazole) and then transferred them to galactose to induce a DSB. In galactose, cells were either retained in the cell cycle arrest (blocked cells) or released to freely cycle (synchronized

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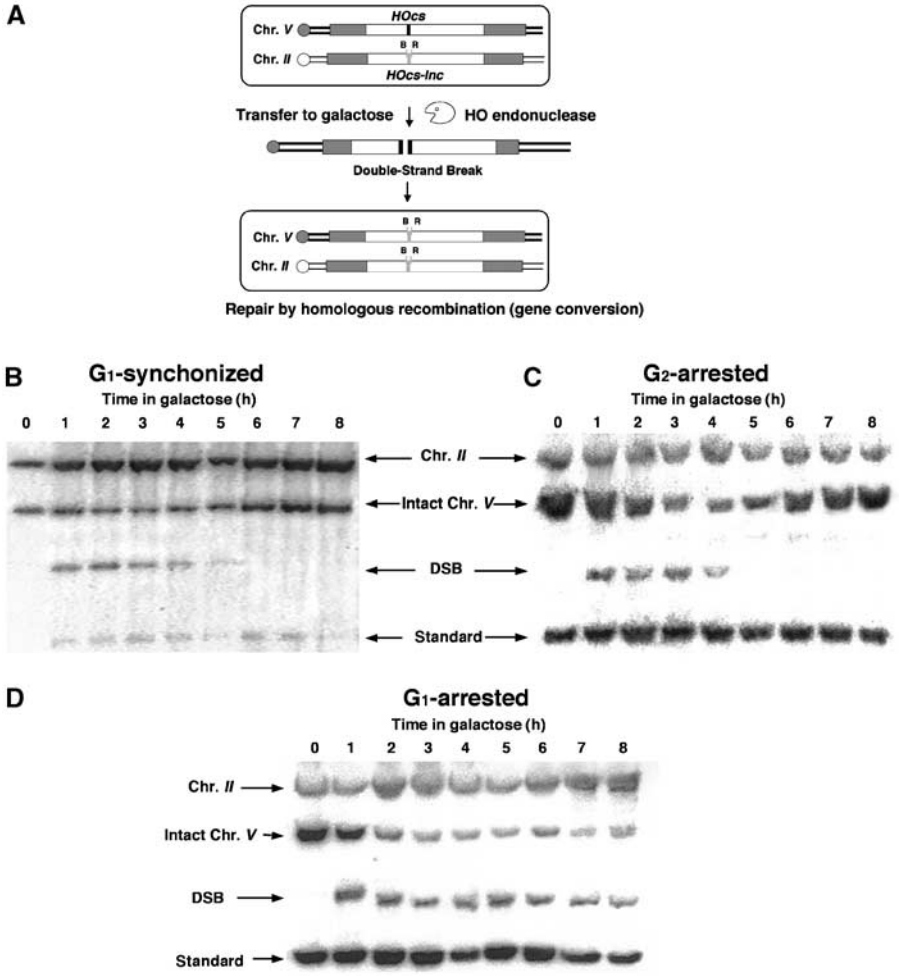

Figure 1 G_1-arrested cells are unable to repair a DSB. (**A**) Schematic representation of our experimental system. Open rectangles represent the *ura3* alleles on chromosomes II and V. A black box represents the *HOcs*; a grey box depicts the inactive *HOcs-inc* flanked by the *Bam*HI (designated B) and *Eco*RI (designated R) restriction sites. Transfer of the cells to galactose-containing medium results in a DSB that is repaired by homologous recombination. (**B**) Southern blot analysis of cells synchronized in G_1 with alpha-factor and released from the arrest at $t = 0$ in medium containing galactose. The genomic DNA was digested with *Cla*I and probed with a 1.2 kb fragment containing the *URA3* gene (open rectangles in (A)). The donor sequences on chromosome II, the intact chromosome V and one of the two bands created by the DSB are shown. A probe complementary to *LEU2* sequences on chromosome III served as a loading standard. (**C**) Southern blot analysis of DNA from cells arrested at G_2 with nocodazole and transferred to galactose-containing medium in the presence of nocodazole. (**D**) Southern blot analysis of DNA from cells arrested at G_1 with alpha-factor and transferred to galactose-containing medium in the presence of alpha-factor.

cells). The repair kinetics were monitored by Southern blot analysis (Figure 1B–D). Although chromosome V was broken with similar timing in all conditions, the ability of cells to undergo gene conversion was strikingly different at different stages of the cell cycle. Cycling cells synchronized at G_1 or cells blocked at G_2 repaired and re-accumulated intact chromosome V. Cells blocked at G_1 did not repair the DSB. When arrested at this stage, the broken chromosome persisted throughout the experiment (8 h); nevertheless, approximately 40% of chromosome V was visualized as intact (Figure 1D). This is in marked contrast to what is observed in strains lacking components of the recombination machinery, in which the chromosome is broken and undergoes degradation, leaving no intact chromosome V (Aylon *et al*, 2003). This suggests that, in cells blocked at G_1, there may be an alternate repair mechanism that can reinstate the HOcs.

The relative amount of intact chromosome V-carrying polymorphic restriction sites represents the portion of the population that has undergone homologous repair (Aylon and Kupiec, 2003; Aylon *et al*, 2003). Cells blocked and retained at the G_1 phase of the cell cycle did not undergo gene conversion (only after prolonged incubation with alpha-factor a minimal amount of homologous repair is detected, probably reflecting a small fraction of cells that adapted to alpha-factor and escaped cell cycle blockage). In contrast, G_2-arrested cells transferred the *Bam*HI site and repaired the DSB in a highly efficient manner (Figure 2A and B). These results indicate that inability to undergo homologous recombination is not a function of imposed cell cycle arrest and that cells need not traverse the S phase with DNA damage in order to activate repair mechanisms. Cells avoid homologous recombination particularly in the G_1 stage of the cell cycle. Consistently, cycling cells induced with a DSB at G_2 completed recombination more rapidly than cells with a DSB at G_1 (Figure 2B).

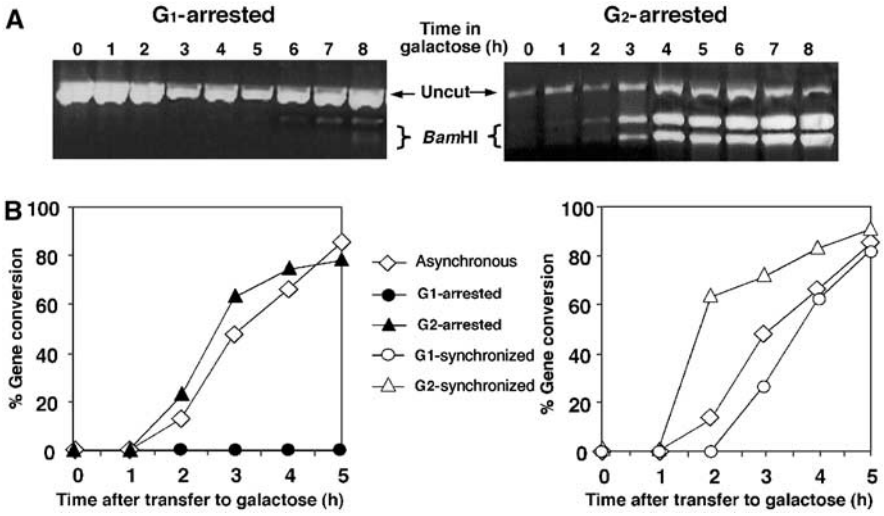

Figure 2 G_1-arrested cells do not carry out gene conversion and cells synchronized at G_1 delay gene conversion. (**A**) PCR-based assay for the detection of gene conversion. The region flanking the site of the DSB was amplified by PCR. The products were digested with *Bam*HI and subjected to electrophoresis. The presence of a *Bam*HI site demonstrates repair by gene conversion. (**B**) Kinetics of gene conversion of MK203 cells at different stages of the cell cycle. The ratio between *Bam*HI-cut and total PCR products was plotted as a function of time.

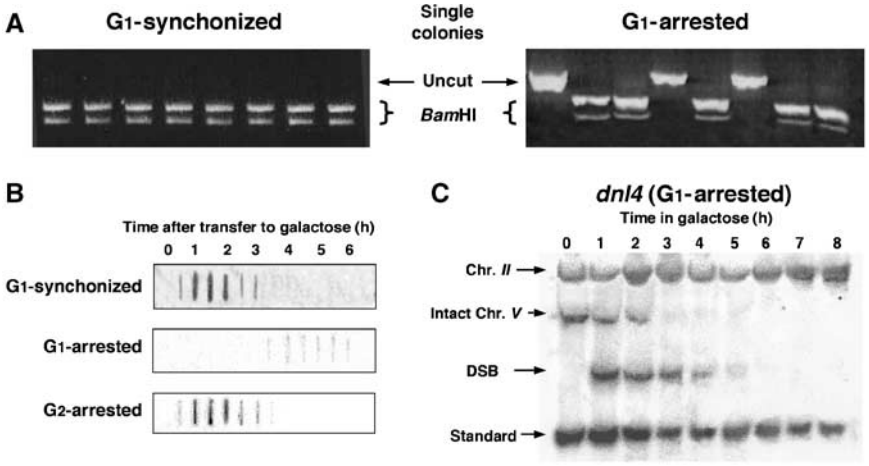

Figure 3 DSB ends remain unresected in G_1-arrested cells and are repaired by NHEJ. (**A**) G1-synchronized and G1-arrested cells were incubated in galactose-containing medium for 6 h and plated on glucose-containing medium. The fate of the broken chromosomes in randomly picked individual colonies was analysed by PCR as described above. (**B**) Nondenaturing slot blot analysis was carried out using a 1.2 kb fragment of the *URA3* gene (open rectangle in Figure 1A). Resected DNA hybridizes to the probe. (**C**) Southern blot analysis of genomic DNA from G_1-arrested *dnl4* cells in galactose.

When plated on medium containing glucose, the HO endonuclease is repressed, and cells can in principle repair the break either by NHEJ or by homologous recombination. Previously, we have defined commitment to gene conversion (CGC) as the stage at which the cells will complete repair by homologous recombination when plated on glucose-containing media (Aylon and Kupiec, 2003). In cycling cells, CGC occurs very rapidly, close to the time of DSB formation, and at least 90 min before actual conversion takes place. CGC probably represents a physical modification of the broken ends (e.g., resection) that precludes end-joining (Aylon and Kupiec, 2003; Aylon *et al*, 2003). Cycling cells committed to gene conversion very early and generated colonies carrying the polymorphic restriction sites (Figure 3A). In contrast, among cells arrested for 6 h at G_1 and released from the arrest by plating on YEPD, as much as 30% of the cells had avoided commitment to gene conversion and retained intact *HOcs* (Figure 3A). Accordingly, 6 h after DSB induction, PCR products obtained from cells blocked at G_1 could be digested neither by *Eco*RI nor *Bam*HI, nor was inaccurate end-joining detected by DNA sequencing (data not shown). This indicates that in G_1-blocked cells, the ends of the broken chromosomes are capable of simple re-ligation for prolonged periods of time after DSB induction, a phenomenon that has been observed previously (Frank-Vaillant and Marcand, 2002).

Using nondenaturative dot-blot analysis and probes complementary to sequences flanking the DSB, the single-stranded DNA (ssDNA) repair intermediate can be measured (Aylon and Kupiec, 2003; Aylon *et al*, 2003). Both G_1-synchronized and G_2-arrested cells rapidly accumulated ssDNA

(Figure 3B). In contrast, in G_1-blocked cells, after prolonged incubation in galactose medium, ssDNA was detected only at extremely low levels. This indicates that DSB ends do not undergo processing at the G_1 phase of the cell cycle.

Since unprocessed DSB ends are ideal substrates for end-joining, NHEJ is a plausible candidate pathway for DSB repair. The *DNL4* gene is central to the NHEJ process. Unlike many other genes involved in end-joining, *DNL4* has no additional phenotypes (telomeric or silencing defects) that might indirectly influence a cell's ability to repair a DSB (Teo and Jackson, 1997). We examined the repair kinetics of a G_1-blocked or freely cycling *dnl4* strain. Southern blot analysis of cycling cells showed that the efficiency of repair was indistinguishable between wild-type (wt) and *dnl4* strains (data not shown). In contrast, in G_1-arrested ligation-deficient cells, no intact chromosome V could be detected by 4 h after transfer to galactose (compare Figures 3C and 1D). This implies that the intact chromosome V that persists in G_1-blocked wt cells is reconstituted through a mechanism that relies on Dnl4. Moreover, in the absence of both homologous recombination and ligation by the NHEJ machinery, the DSB ends were no longer stable and were degraded. When homologous recombination is impeded, the NHEJ pathway may have a competitive advantage and predominate.

Haploid yeast cells occur in two mating types, 'a' and 'alpha'. Through a highly controlled process, yeast cells at G_1 undergo mating type switch (Haber, 1998). This process is induced by a single DSB created by the HO enzyme at the *MAT* locus, and is accomplished by directional gene conversion. Despite the similarities, genomic DSBs activate an emergency repair and checkpoint response that is not normally elicited in the developmentally controlled *MAT* switching process (Pellicioli *et al*, 2001). Our MK203-derived strains are unable to undergo switching because the endogenous *HOcs* has been mutated (*MATa-inc*). We reinstated the intact *MATa* allele and followed mating type switching with PCR primers specific for *MATalpha*. Despite the G_1 block, cells were able to undergo *MAT* switching (Figure 4A), albeit with slightly delayed kinetics compared to asynchronous cultures (data not shown). Evidently, cells are enzymatically capable of performing gene conversion at G_1, but genomic recombinational repair is restricted.

Genomic DSBs trigger a cell cycle arrest in a checkpoint-dependent manner. The DNA damage checkpoint also affects repair efficiency and pathway choice (Aylon and Kupiec, 2003). Deletion of *RAD9* and *RAD24* severely impairs the DNA damage response (de la Torre-Ruiz *et al*, 1998; Aylon and Kupiec, 2003). However, checkpoint activity is not required to prevent homologous recombination in G_1, since *rad9 rad24* mutants blocked at G_1 also did not undergo gene conversion (Figure 4B).

The inability to carry out homologous recombination in G_1 might have been particular to ectopic gene conversion. In diploids, allelic recombination between homologous chromosomes can occur, thus providing the means for homologous recombination at all stages of the cell cycle. Allelic recombination is also possible in genetically manipulated haploid yeast cells supplemented with an additional homologous chromosome (disomal strains). In these strains, gene conversion results in transfer of either *Nco*I from the allelic chromosome V or *Bam*HI from the ectopic sequence on chromosome

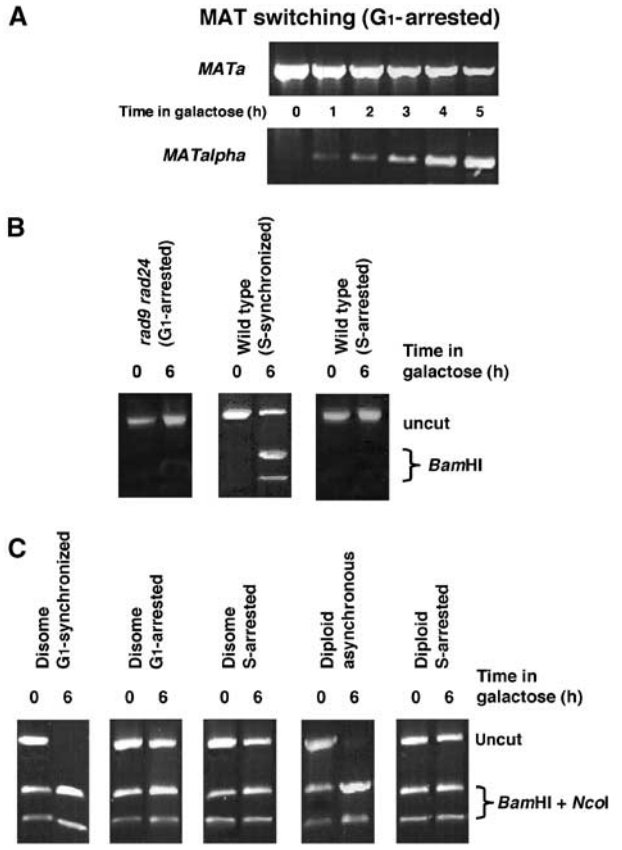

Figure 4 G_1-arrested cells are able to carry out MAT switching; the inability to repair DSBs by homologous recombination in G_1 is independent of checkpoint response and ploidy. (**A**) A *MATa* derivative of MK203 was retained at G1 (alpha-factor) and monitored for the ability to switch mating type with *MATalpha*-specific PCR primers. (**B**) PCR assays for gene conversion were carried out for *rad9 rad24* synchronized or arrested in G_1, and wt cells synchronized or arrested in S-phase with HU. (**C**) Disomic and diploid strains are unable to carry out gene conversion when arrested at G1 (alpha-factor) or S (HU). PCR was carried out as in (B) and PCR products were digested with *Bam*HI and *Nco*I. The presence of uncut product at $t = 6$ h indicates that no gene conversion occurred.

II. Since the allelic donor sequences are identical except the *HOcs* polymorphism, at $t = 0$ h, half of the products can be digested with *Nco*I. In disomal strains synchronized at G_1 and released, all cells had undergone either allelic or ectopic gene conversion by 6 h after transfer to galactose. In contrast, disomal cells blocked at G_1 with alpha-factor executed neither ectopic nor allelic recombination (Figure 4C). Diploid a/alpha yeast cells do not respond to alpha-factor. To investigate the ability of diploid cells to carry out homologous recombination while blocked in the cell cycle, we used hydroxyurea (HU), a potent inhibitor of the enzyme ribonucleotide reductase, which arrests cells at the beginning of S phase (Pellicioli *et al*, 1999). No homologous recombination could be observed in HU-treated haploid or diploid cells (Figure 4B and C).

Taken together, these results indicate that the inhibition of recombination in G_1/early S phase occurs irrespective of recombination pathway or cell ploidy. Although it has been recently reported that HU-arrested cells retain as much as 80% of their G_1 levels of dNTPs (Koc *et al*, 2004), even such a mild reduction in dNTP levels by HU might have had a direct

effect on recombination. In order to avoid this possibility, we carried out additional experiments with cells arrested before DNA replication. Moreover, by exploiting the wide variety of yeast mutants that have been characterized previously, we were able to determine whether recombination is dependent on the presence of duplicated DNA molecules or on other events that take place during S phase.

At restrictive temperatures, *cdc4-1* mutants halt both replication and cell cycle progression through CDK inactivation (Goh and Surana, 1999), whereas *cdc7-4* mutants prevent the initiation of DNA replication but are unable to prevent later cell cycle processes such as G_2 progression and mitosis (Tercero *et al*, 2003). Replication mutants *cdc4-1* and *cdc7-4* incubated at 37°C for 3 h arrested with a 1C DNA content (data not shown and Figure 5A). The cultures were then transferred to galactose at either the permissive (25°C) or restrictive temperature (37°C). At the permissive temperature, both strains repaired the broken chromosome with wt kinetics (Figure 5B and C). As could be predicted for a strain that arrests at the G_1/S transition, *cdc4-1* mutants at the restrictive temperature did not undergo gene conversion. In contrast, a large portion of the *cdc7-4* mutant population (approximately 40%) completed gene conversion, even at the restrictive temperature (Figure 5B). The ability of *cdc7-4* cells to accomplish homologous repair in the absence of DNA replication suggests that competency for homologous recombination is controlled by the sequential action of CDK–cyclin complexes and not by the presence of duplicated DNA.

Sic1 is a potent inhibitor of the Clb–CDK (Mendenhall, 1993; Peter and Herskowitz, 1994). We overexpressed *SIC1* to inactivate the CDK (Peter and Herskowitz, 1994) in pre-replicative or post-replicative cells. Overexpression of *SIC1* in both situations prevented repair by gene conversion (Figure 5C), reconfirming that Clb–CDK activity, and not the presence of duplicated DNA, is essential for homologous recombination. Consistently, inactivation of the CDK in *cdc28-4* mutants at the restrictive temperature also prevented recombinational repair (Figure 5C).

In order to investigate the mechanism that controls homologous recombination during the cell cycle, we used nondenaturative dot-blot analysis to monitor ssDNA end resection in the different strains. Whereas *cdc7-4* cells processed DSBs into ssDNA resection intermediates, all of the strains lacking Clb–CDK activity (*cdc28-4*, *cdc4-1* and wt cells overexpressing *SIC1*) failed to undergo resection (Figure 5D). We thus conclude that Clb–CDK activity promotes homologous recombination by controlling the resection step of DSB repair.

Earlier studies in yeast have provided evidence that recombination proficiency is in fact independent of cell cycle stage (Fabre *et al*, 1984; Galli and Schiestl, 1998). However, most of these experiments were based on the selection of rare recombination products, which do not represent the majority of the cell population. Differential damage sensitivity of synchronized, cycling cells has been used to infer a preference for homologous recombination in G_2 also in mammalian systems (Takata *et al*, 1998). In our experiments, a single, defined DSB was created uniformly in each cell and repair was monitored in the entire population. We were also able to retain the population at the specific cell cycle stages tested, and monitor the repair in real time while the cells remained arrested. Our experiments thus have uniquely defined the relationship between homologous repair, CDK activity and DNA replication in a eukaryotic system.

The ability of yeast cells to carry out the developmentally controlled gene conversion required for mating type switch at the G_1/S stage demonstrates that the enzymatic machinery required for homologous recombination is present in the cells. Further work is required to find out the mechanism by which a DSB at *MAT* is recognized and processed by homologous recombination, whereas DSBs at other regions of the genome are not.

The lack of end processing has several important consequences. It preserves an appropriate substrate for NHEJ in G_1 and defers initiation of recombination until G_2, during which homologous sequences are present in the form of sister chromatids. In addition, since checkpoint activation is proportional to the extent of resected ssDNA (Melo *et al*, 2001; Zou and Elledge, 2003), the DNA damage checkpoint may not be triggered in G_1-arrested cells (Pellicioli *et al*, 1999, 2001).

Our results clearly show that lack of Clb–CDK activity in G_1 cells prevents homologous recombination by inhibiting ssDNA resection. This may be a direct effect (Clb–CDK directly phosphorylating a target protein involved in resection). An alternative model suggests that Clb–CDK may be needed to prevent end-joining, thus allowing the homologous recombination machinery to compete more efficiently for the broken ends. Future work will be aimed at identifying the CDK target(s) important for resection.

Restriction of homologous recombination from the G_1/S phase of the cell cycle by lack of Clb–CDK activity has clear adaptive advantages for all eukaryotes. For organisms such as *S. cerevisiae*, which have a compact genome with a high gene density, it may be advantageous to rely upon homologous recombination, an error-free mechanism of repair. In contrast, in mammalian cells, inexactly ligated sequences are less likely to affect functional genes. Furthermore, the presence of numerous repeated sequences in the mammalian genome could make homologous recombination dangerous. Accordingly, DNA damage in the form of DSBs primarily elicit G_1 checkpoints in mammalian cells and G_2 checkpoints in yeast (Bartek *et al*, 1999). Our results provide a cellular mechanism for the control of eukaryotic DSB repair pathway choice.

Materials and methods

Yeast strains

All the yeast strains used in this study are isogenic derivatives of strain MK203 (*MATa-inc ura3::HOcs lys2::ura3::HOcs-inc ade3::GALHO ade2-1 leu2-3,112 his3-11,15 trp1-1 can1-100*) (Aylon *et al*, 2003), a derivative of W303. The *ura3::HOcs* allele on chromosome V was created by inserting a 39 bp oligonucleotide at the *Nco*I site of the *URA3* gene. The *ura3HOcs-inc* allele on chromosome II was created by inserting a 1.2 kb *ura3HOcs-inc Hind*III fragment at a *Hpa*I site within *LYS2* sequences, as described (Inbar and Kupiec, 1999).

Deletion of genes was obtained by transformation of MK203 with a PCR product produced on the appropriate strain from the *Saccharomyces* Genome Deletion Project array.

W303 strains of the *cdc4-1*, *cdc7-4* and *cdc28-4* alleles were kindly provided by J Diffley and Y Kassir. MK203 derivatives carrying these alleles were generated by genetic crosses. The plasmid carrying *GAL-SIC1* was generously provided by D Kornitzer and was introduced into MK203 by transformation. All chromosomal configurations were verified by Southern blot or PCR analysis after transformation.

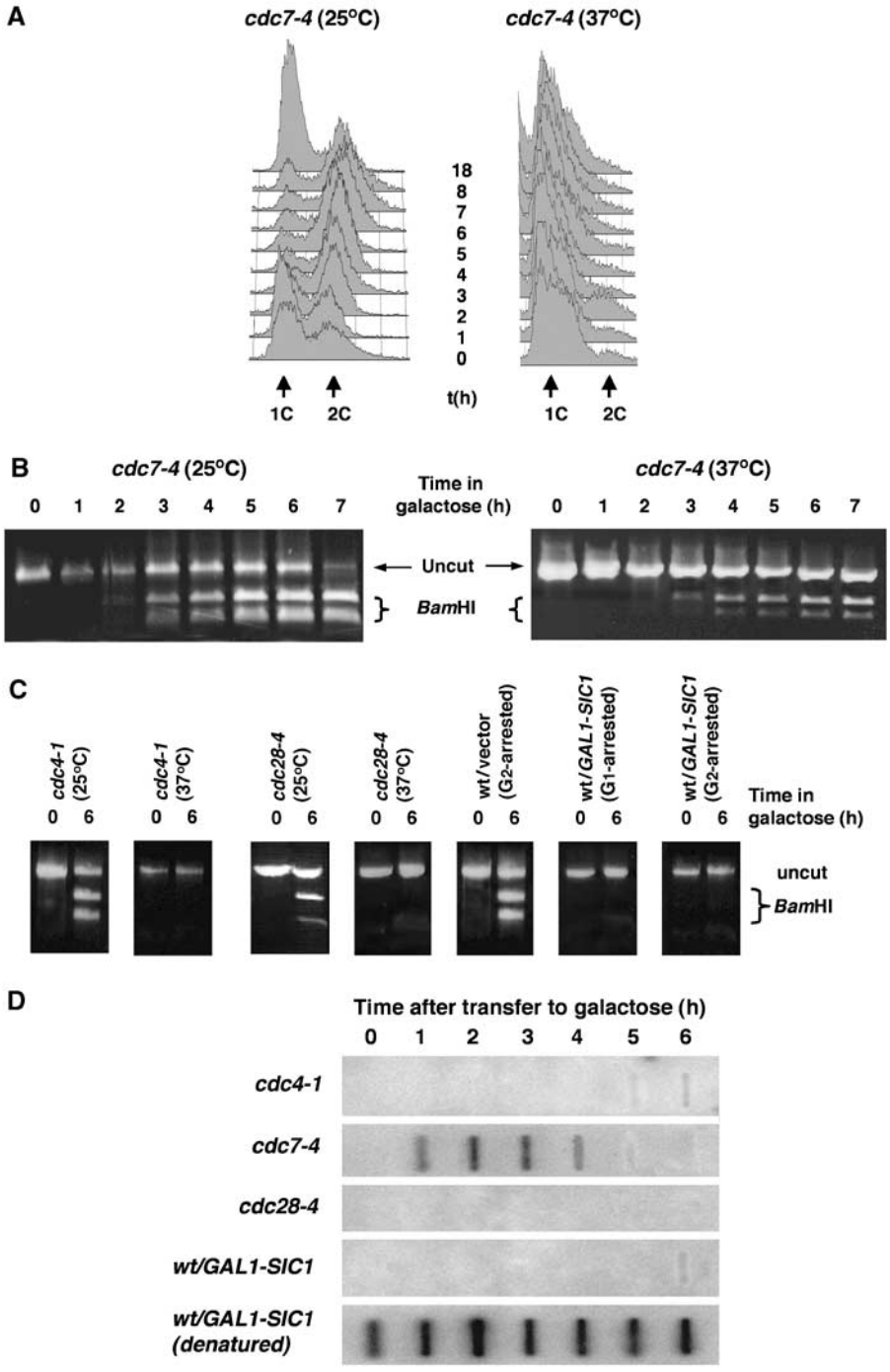

Figure 5 Homologous recombination is dependent on active Clb–Cdk and not on DNA replication. (**A**) *cdc7-4* cells were arrested at the restrictive temperature and then incubated in galactose-containing medium at the permissive (25°C) or restrictive (37°C) temperatures. FACS analysis of the *cdc7-4* cell culture confirmed that cells did not undergo DNA replication at the restrictive temperature. (**B**) PCR assays for gene conversion in *cdc7-4* cells at the permissive and restrictive temperatures. (**C**) PCR assays for gene conversion were carried out for *cdc4-1* and *cdc28-4* cells at the permissive and restrictive temperatures, wt G_2-arrested with vector only, or G_1- or G_2-arrested with *GAL-SIC1* plasmid. (**D**) Nondenaturing slot blot analysis of *cdc4-1*, *cdc7-4*, *cdc28-4* and wt cells overexpressing *SIC1*. As a positive control for hybridization, equivalent DNA samples were blotted after DNA denaturation (only wt/*GAL-SIC* is shown here).

Disomic strains were created by crossing cycloheximide-resistant papillae of MK203 *bar1*::*LEU2* cells to mutants carrying a *kar1-1* mutation (provided by Giora Simchen). Heterokaryons were plated on medium to select for the following phenotype: Cyh^r Leu^+ Ura^+, and tested for additional markers (Lys^- Trp^- Ade^- His^- CAN^s), mating ability and high levels of papillation of 5-FOA and CAN medium, indicative of loss of heterozygozity of markers on chromosome V. In addition, heterozygosity for *URA3* was verified using PCR, so that disomic strains carried one *URA3* allele and one *HOcs* allele on each chromosome V (in addition to a *ura3:: HOcs-inc* on chromosome II).

Diploid MK235 strain was generated by crossing MK203 with MK228 (a *LYS2 URA3* derivative of W303).

Media and growth conditions

S. cerevisiae strains were normally grown at 30°C. Temperature-sensitive strains were tested at 25°C (permissive) or 37°C (restrictive) temperatures. Standard YEP medium (1% yeast extract, 2% bacto peptone) supplemented with 3% glycerol (YEPGly), 2% galactose (YEPGal) or 2% dextrose (YEPD) was used for non-selective growth. 1.8% Bacto-Agar was added for solid media. For cell cycle arrest, 3.4 μM alpha-factor, 100 mM HU and 15 μg/ml nocodazole were used for G_1, S and G_2 block, respectively.

DSB induction experiments

Single colonies were resuspended in rich YEPGly medium, grown to logarithmic phase, centrifuged and resuspended in YEPGal. At timely intervals, samples were plated on YEPD plates to score viability and commitment to gene conversion, and DNA was extracted and subjected to the different assays. At least three independent experiments were carried out for each condition analyzed.

Fluorescent activated cell sorter (FACS) analysis

Samples of 10^7 cells were harvested and fixed in 1 ml 80% ethanol. Cells were washed twice with 1 ml 50 mM Tris–HCl (pH 8) and resuspended in the same buffer with 2 mg/ml RNAse A. Following overnight incubation at 37°C, the samples were washed twice with 1 ml 50 mM Tris–HCl (pH 8), resuspended in 50 μl 20 mg/ml Proteinase K and incubated for 1 h at 37°C. After another wash and brief sonication, 3 μl of 4 mg/ml propidium iodide was added and cells were appropriately diluted and analyzed.

Southern blot analysis

Southern blot analysis was carried out as described previously (Inbar and Kupiec, 1999).

PCR assays

Genomic DNA (5 ng) was amplified in each sample. Taq polymerase was used in standard reaction conditions. Primers OI9 and OI10 are complementary to sequences flanking the DSB on chromosome V. Primers MATa and MAT distal detect *MATa* configuration; MATalpha and MAT distal detect *MATalpha* configuration. Sequences are provided below:

OI9: 5′ TTTTGCGAGGCATATTTATGGTGAAGG 3′
OI10: 5′ TGTTACTTGGTTCTGGCGAGGTATTGG 3′

MATa: 5′ AAATAAACGTATGAGATCTA 3′
MATalpha: 5′ GCAGCACGGAATATGGGACT 3′
MAT distal: 5′ ATGTGAACCGCATGGGCAGT 3′

Nondenaturing slot blots

Equal amounts of nondenatured genomic DNA were directly spotted on nylon Hybond + filters, or denatured by boiling and immediately spotted on filters. All denatured samples undergo hybridization, whereas hybridization of nondenatured DNA indicates that resection had occurred. A 1.2 kb *Hind*III *URA3* fragment was used as a probe for ssDNA. Hybridization and exposure were carried out as in Southern blot analysis.

Survival

Survival was assayed by plating samples of cells grown on liquid YEPGal, onto YEPD plates at different times during a DSB induction experiment.

Commitment to gene conversion

At different times after transfer to galactose, cells were plated onto YPED and incubated 3 days at 30°C. In all, 30 individual colonies were subjected to either Southern blot analysis or PCR, to assay the transfer of information in individual colonies. The PCR reactions consisted of one cycle of 5 min at 96°C, followed by 30 cycles at standard conditions. The DNA thus obtained was then digested with *Bam*HI or *Eco*RI and subjected to electrophoresis.

Quantitation of results

Southern blots and ethidium bromide-stained agarose gels were quantified using the TINA (v. 2.1) and NIHimage (v. 1.62) computer programs, respectively.

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