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Regulation of Repair Choice:

Cdk1 Suppresses Recruitment of End Joining Factors at DNA Breaks

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Abstract

Cell cycle plays a crucial role in regulating the pathway used to repair DNA double-strand breaks (DSBs). In *Saccharomyces cerevisiae*, homologous recombination is primarily limited to non-G_1 cells as the formation of recombinogenic single-stranded DNA requires *CDK1*-dependent 5′ to 3′ resection of DNA ends. However, the effect of cell cycle on non-homologous end joining (NHEJ) is not yet clearly defined. Using an assay to quantitatively measure the contributions of each repair pathway to repair product formation and cellular survival after DSB induction, we found that NHEJ is most efficient at G_1, and markedly repressed at G_2. Repression of NHEJ at G_2 is achieved by efficient end resection and by the reduced association of core NHEJ proteins with DNA breaks, both of which depend on the *CDK1* activity. Importantly, repression of 5′ end resection by *CDK1* inhibition at G_2 alone did not fully restore either physical association of Ku/Dnl4-Lif1 with DSBs or NHEJ proficiency to the level at G_1. Expression of excess Ku can partially offset the inhibition of end joining at G_2. The results suggest that regulation of Ku/Dnl4-Lif1 affinity for DNA ends may contribute to the cell cycle-dependent modulation of NHEJ efficiency.

Keywords

Double strand break; End joining; Repair choice; Cell cycle

1. Introduction

Mutations, especially gross chromosome rearrangements, are frequent in cells from cancer patients, and often the consequence of failed and/or improper repair of DNA lesions (reviewed in [1]). Among DNA lesions, double-strand breaks (DSBs) pose the most serious challenge as the un-repaired DSB is highly mutagenic and frequently lethal. Two highly efficient repair pathways have evolved to deal with this type of lesion: homologous recombination and non-homologous end joining (NHEJ) [2,3]. Homologous recombination (HR) uses an undamaged template, often found in the sister chromatid or homologous chromosome to restore chromosome integrity [3]. NHEJ, in contrast, restores chromosome integrity by simple ligation of the broken chromosome ends back together following appropriate end processing [4,5]. Because end processing, catalyzed by a collection of polymerases and nucleases, inevitably

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alters terminal sequences, NHEJ is often considered more mutagenic than HR [4,6]. However, HR between ectopic templates can in principle lead to chromosome rearrangement type mutations [7]. The prudent choice of repair pathway will thus greatly reduce the mutation rate and improve the overall cellular fitness [3]. The fact that specific types of DSB repair such as V(D)J recombination, class switch recombination (CSR) and meiotic recombination exclusively rely on one of these two repair pathways also lends additional support to cellular regulation of repair pathway choice [8-10]. How a cell imposes the use of a specific repair pathways has begun to emerge with a finding of a role of the post-cleavage Rag1/Rag2 complex during V(D)J recombination [9] and end processing in yeast [11,12].

The cell cycle has long been recognized as a crucial factor for determining repair pathway choice [3]. Early studies in vertebrates showed that NHEJ-deficient *scid* cells and *ku70*$^{-/-}$ chicken DT40 cells were hypersensitive to ionizing radiation only during G_1 and early S phase of the cell cycle, whereas HR-defective *rad54*$^{-/-}$ cells were radiation sensitive at late S/G_2 [13,14]. In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, recombination is also tightly linked to cell cycle such that recombination between sister chromatids predominates at S/G_2 [11,12,15,16]. When sister chromatids are unavailable in G_1, recombination is minimized to prevent the loss of heterozygosity or chromosome rearrangements [17]. Recombination in yeast is suppressed at G_1 because end resection, the key early step that produces the 3′ single-stranded DNA (ssDNA) essential for strand invasion [18], depends on the Cdk1 kinase activity [11,12,16]. Recently, phosphorylation of Sae2 or its mammalian counterpart CtIP, at its carboxyl terminus by Cdk1 has been implicated in cell cycle dependent regulation of DSB processing and hence repair choice during the cell cycle [19,20]. The strand invasion step during gene conversion, where Rad51-coated presynaptic filaments search and anneal to the homologous template, is also controlled by the cell cycle [11]. Finally, cell cycle controls repair pathway choice by modulating expression of recombination proteins. In mammals, the expression level of Rad51 and Rad52 is the lowest at G_0/G_1 and gradually increases during S/G_2/M [21], and in *S. pombe*, the resection facilitator Ctp1 protein (Sae2 in *S. cerevisiae*) is not expressed at G_1 [22]. An equivalent expression pattern of Sae2 during the cell cycle is not detected in *S. cerevisiae* [23], however, suggesting that this type of regulation might be species specific.

While evidence has accumulated for cell cycle-dependent regulation of HR, it is not clear whether the cell cycle has a similar role in regulating NHEJ. In fact, considerable uncertainty exists as to the role of NHEJ in DSB repair during the S and G_2 phases of the cell cycle. DNA ends with extensive 5′ degradation would not likely be favorable substrates for NHEJ [11,24, 25], suggesting that Cdk1-dependent resection of DNA ends might suppress NHEJ at late S/G_2. Alternatively, efficient HR at S/G_2 may simply out-compete NHEJ and cells may channel DNA breaks preferentially to HR despite the lack of a clear decline in NHEJ efficiency at this cell cycle stage [26,27].

In this study, we examined the effect of cell cycle on the repair of DNA breaks by NHEJ and described a biochemical basis for NHEJ suppression at S/G_2 that operates independently of HR events. We have uncovered a role of Cdk1 in opposing the association of Ku/Dnl4-Lif1 with DNA breaks and thus in discouraging commitment to NHEJ at S/G_2. Cdk1-dependent inhibition of NHEJ factor recruitment at DNA breaks is distinct from Cdk1's role in recombination and end processing, and represents a novel mode of pathway choice control for DSB repair.

2. Materials and Methods

2.1. Strains

Strains used in these studies are listed in Table 1. JKM161 and its mutant derivatives bearing a single *HML*α donor were used to measure HR and NHEJ efficiency, while donorless JKM179 derivatives were used to detect resection and the enrichment of repair proteins at a DNA break by ChIP. Yeast cell synchrony was achieved by incubation with 10 μg/ml of α factor (G_1) or 15 μg/ml of nocodazole (G_2) for 4 h prior to HO induction, and confirmed by FACS analysis.

2.2 Survival rate determination and detection of repair products

HO breaks were induced by addition of galactose [final conc. 2% (w/v)] to cells grown in YEP-glycerol pre-induction media. Following 1 h incubation in the galactose-containing medium, cells were plated onto YEP-dextrose plates and the number of colonies was counted after 3 days at 30°C. The survival frequency was calculated by normalization with the number of colonies obtained without galactose addition. To detect HR or NHEJ products, genomic DNA extracted from time course samples was digested with EcoRV, separated by an agarose gel (1%), and transferred to nylon membrane (Hybond N+). The blot was probed with a 1 kb ^{32}P-labeled *MAT* distal probe that recognizes the 3.4 kb (*MAT*α) or the 5.0 kb (*MAT***a**) fragment containing the HO cleavage site on yeast chromosome III. The levels of HR and NHEJ repair products were determined after normalization to a *HIS3* sequence that served as an internal loading control.

2.3. Chromatin immunoprecipitation (ChIP)

ChIP assays were performed as described previously [28]. After immunoprecipitation and crosslink reversal, purified DNA was analyzed by real time quantitative PCR using multiple sets of primers that anneal 0.2-kb, 1-kb, and 5-kb from the DSB, as well as primers specific for the *PRE1* gene situated on chromosome V as a control. The antibodies for RPA were a generous gift from Dr. S. Brill.

2.4. Ligation mediated PCR assay

Ligation mediated PCR was performed as described [25], except that real time quantitative PCR was used instead of radiolabeled PCR. Briefly, genomic DNA was extracted by a standard glass bead protocol, and subjected to ligation with a linker containing a 4 base overhang complementary to the distal side of the HO cut site. Only the unprocessed ends could be ligated with the linker. PCR was carried out using a pair of primers recognizing DNA sequence distal to the HO site and the adaptor.

2.5. Immuno blot

Whole cell extracts were prepared as described [29]. Proteins were separated by SDS-PAGE and transferred to PVDF membrane. The TAP fusion proteins were detected by Peroxidase-Anti-Peroxidase (PAP) soluble complex (Sigma). Phosphorylation of the B subunit of DNA polymerase α, a marker for Cdk1/Clb activity, was detected by monoclonal antibody 6D2 [11](a gift from Dr. Achille Pellicioli).

3. Results

3. 1. NHEJ is repressed at G_2

To assess the effect of cell cycle on DSB repair, a DSB was induced at the *MAT* locus of strain JKM161 using a galactose-inducible HO gene integrated at the *ADE3* locus. Because this strain lacks *HMR***a**, *HML*α is the sole template for the repairing the HO break (Fig. 1a)[28,30]. In JKM161, repair of HO-induced break by HR is associated with mating type switching from

a to α, while NHEJ will retain the mating type as **a**. HO cleavage is very efficient (>99% after 1 h induction) and almost all survivors repair the DSB by one of the two repair mechanisms [28,30].

When HO was induced for 1 h in logarithmically growing wild type cells, almost all cells survived the break. Among survivors, 85% repaired the break by HR (α mating type), whereas 15% repaired the break by NHEJ (**a** mating type) (Fig. 1c). Deletion of *RAD52* completely eliminated HR leaving only ~10% survivors, all of which were repaired by NHEJ (**a** mating type). In contrast, deletion of *YKU70* had no influence on the survival frequency apparently because more survivors form by HR (switched to α type), consistent with the previous reports that HR is enhanced in the absence of Yku70 [28,31-33](Fig. 1c). We also used Southern blot hybridization to measure the amount of repair product generated as a function of time after HO expression (Figs. 1b, 1d). The amount of HR and NHEJ products in the Southern blot analysis was in an excellent agreement with the survival data in wild type, *yku70Δ* and *rad52Δ* strains.

The effect of cell cycle on the DSB repair pathway choice was determined by arresting cells at G_1 with α factor or at G_2 with nocodazole treatment, and then inducing HO for 1 h before turning it off by addition of glucose. The HO enzyme is rapidly (a half life of ~10 min) degraded by the ubiquitin-26S proteasome system after glucose addition [34]. Following 3 hours of incubation in glucose containing media at the indicated cell cycle stage, cells were then plated onto glucose containing plates to score the number of survivors (Fig. 1c). At G_1 far less of the survivors (41% vs. 85% in asynchronous cells) repaired the DSB by HR, while more repaired the break by NHEJ (35% vs. 15% in asynchronous cells; Fig. 1c). In contrast, over 85% of G_2 cells repaired the break by HR but a mere 7% did so by NHEJ (Fig. 1c). Detection of repair products by Southern blot hybridization confirmed this result; over 25% of the G_1 vs. only 5% of the G_2 arrested cells repair the DSB by NHEJ, whereas 10% of the G_1 vs. 95% of the G_2 cells repaired the break by HR (Figs. 1b, 1d). The results confirm the previous reports that HR is less efficient at G_1 than G_2 [11,12,16], and further demonstrate that NHEJ is less efficient at G_2 than G_1. The results are highly congruent with the reduced NHEJ at S/G_2 previously observed using a plasmid based NHEJ assay in cells synchronously progressing through the cell cycle [35].

3.2. NHEJ repression at G_2 does not depend on homologous recombination

NHEJ and HR likely compete for the same DSB substrate [3] and therefore the reduced NHEJ at G_2 may simply reflect the increased effectiveness of HR at this stage of the cell cycle. To address this issue, we determined the NHEJ efficiency in the G_2 arrested *rad52*Δ cells by scoring the number of survivors with the **a** mating type after HO expression. We also quantitated the NHEJ or HR product using Southern blot hybridization. We also examined, the effect of NHEJ on HR at G_1 by measuring the HR efficiency in the G_1 arrested *yku70*Δ cells. We found that deletion of *YKU70* dramatically improved HR at G_1, suggesting that the reduced HR at G_1 is partly due to the elevated NHEJ at this cell cycle stage (Fig. 1b, 1d). In contrast, deletion of *RAD52* did not increase the amount of NHEJ product nor the number of *MAT***a** survivors, indicating that HR does not compete with NHEJ at G_2 (Figs. 1b, 1c, and 1d). Deletion of *HML*α in JKM161 to eliminate the only remaining HR template also did not influence the NHEJ frequency at G_2 (data not shown). Together, we conclude that NHEJ repression at G_2 is distinct from efficient HR at this stage of the cell cycle.

3.3. Recruitment of NHEJ proteins to DSBs is suppressed at G_2

The recruitment of enzymes to the proper DNA substrate is an active process in many DNA transactions including transcription and DNA replication and is frequently used to control the corresponding enzymatic reactions. To elucidate the molecular basis of the cell cycle dependent regulation of NHEJ, we used ChIP to examine if core NHEJ proteins (Yku, Lif1, and Mre11)

are recruited to DSBs differently at G_1 and G_2 in a donorless yeast strain. We found that all three NHEJ proteins were enriched at the HO break more markedly at G_1 than G_2 (Fig. 2). Reduction in recruitment of NHEJ proteins at the DSB at G_2 is due neither to protein level decline at this cell cycle stage as the amount of Yku, Mre11 and Lif1 proteins remained constant at both G_1 and G_2 with or without DNA damage (Supplemental Fig. 2 and data not shown) nor to the presence of active HR at G_2 (Supplemental Fig. 3; the slight reduction in the recruitment of YKu at a DSB in *rad52*Δ may be attributed to reduced HO induction efficiency in this mutant). Previously, both Rad51 and Rad52 were shown to be recruited to a DSB more efficiently at G_2, consistent with the suppression of HR at G_1 [11,12,16,36]. Together, these results suggest that the effect of cell cycle on DSB repair pathway choice may at least partly depend on the efficient recruitment of the corresponding repair proteins to the site of DNA breaks.

3.4. Recruitment of Ku at DSBs is not controlled solely by 5′ end resection

A key question is how the cell cycle modulates the recruitment of recombination and NHEJ factors to DSB sites. Cdk1-dependent resection of DNA ends produces single stranded DNA near a DNA break at the S/G_2 phase of the cell cycle [11,12], and HR proteins such as RPA and Rad51 bind preferentially to ssDNA at S/G2. We reasoned that core NHEJ proteins, Yku70/80, Mre11/Rad50/Xrs2, and Dnl4/Lif1, might bind DNA ends more efficiently at G_1 because of their stronger affinity for unprocessed DNA ends, whereas HR proteins such as RPA and Rad51 bind preferentially to single strand DNA at S/G_2. The model is particularly appealing in explaining the cell cycle dependent enrichment of Yku proteins, as they are already well known for their high affinity to double stranded DNA ends [37]. Likewise, RPA and Rad51 are known single strand DNA binding proteins [38].

To test this hypothesis, we inhibited the Cdk1 activity crucial for end resection either by overexpressing the Sic1 protein, a Cdk1 inhibitor or by expressing Cdc28-as1, a hypomorphic Cdk1 sensitive to the ATP analogue inhibitor 1-NMPP1 [11]. We then monitored the extent of end processing by measuring the level of DSBs carrying an unresected 4 base pair overhang by ligation-mediated PCR (LM-PCR) (Fig. 3a)[25]. As expected, overproduction of Sic1 or inhibition of cdc28-as1 by 1-NMPP1 treatment at G_2 almost completely blocked processing of DNA ends, so that the level of intact DSB ends was comparable to that in G_1, reaffirming that resection was dependent on Cdk1 activity (Fig. 3b).

We then examined the enrichment of NHEJ proteins at the HO break in G_2 when Cdk1was inhibited. We found that Mre11 was enriched to similar levels in G_1 and G_2 when Cdk1 activity was inhibited (Fig. 4a). Similarly, enrichment of Yku70/80 and Lif1 at a DSB was markedly improved when cdc28-as1 activity was inhibited by 1-NMPP1 treatment at G_2 (Fig. 4b and 4c). However, Cdk1 inhibition by overproduction of Sic1 failed to fully de-repress recruitment of Yku and Lif1 to the HO-induced break at G_2, even though resection of DNA ends and formation of ssDNA were reduced to a level indistinguishable from that at G_1 (Fig. 3b, 4b, and 4c). These results suggest that resection is not the sole factor regulating the binding of NHEJ factors to DSBs.

3.5. Repression of NHEJ at G_2 requires Cdk1 activity that modulates end resection and the recruitment of NHEJ factors to DNA break

We next examined whether the elevated recruitment of Yku and Lif1 in the 1-NMPP1 treated *cdc28-as1* strain was accompanied by increased NHEJ of a DSB at G_2 using both the survival assay and Southern blot analysis of repair product formation. In parallel, the level of NHEJ in cells expressing excess Sic1 was determined to define the effect of Cdk1-dependent end processing on NHEJ repression at G_2. We found that only 1-NMPP1-mediated *cdc28-as1* inhibition increased NHEJ. While Sic1 overexpression reduced end resection and HR, it failed

to enhance NHEJ mediated repair of an HO-induced DSB (Fig. 5a and [11]). These results suggest that Cdk1-dependent suppression of NHEJ protein recruitment to a DNA break contributes to the inefficient NHEJ at G_2.

Recruitment of Yku and Dnl4/Lif1 to DNA breaks is mutually dependent [28,39,40]. We expressed excess Yku70 and Yku80 from plasmids harboring the *YKU70* and *YKU80* genes under constitutively active promoters, and asked if expression of excess Ku proteins can override NHEJ repression at G_2 (Fig. 5a). We found that expression of excess Yku proteins partially rescued NHEJ at G_2, increasing NHEJ to a level even higher than that at G_1 when Yku was endogenously expressed. Nevertheless, neither Yku overexpression nor Sic1-mediated repression of end resection fully de-repressed NHEJ at G_2; the NHEJ level at G_2 failed to match that at G_1 under the same conditions (Fig. 5a). The results support a model in which at least two distinct factors contribute to the NHEJ repression at G_2: elevated end resection and reduced association of Yku and/or Dnl4/Lif1 to DNA breaks.

Why do two Cdk1 inhibition methods elicit very different responses to NHEJ factor recruitment and NHEJ repair, even though they are equally efficient in suppressing end resection? To gain insights into this question, we monitored CDK1 activity in cells subjected to each of these two Cdk1 inhibition methods by examining the phosphorylation status of the B subunit of DNA polymerase α, a well-known substrate for the Cdk1 kinase [41]. The data revealed that the 1-NMPP1-treated *cdc28-as1* cells exhibited a far greater inhibition of Cdk1 activity than cells with Sic1 overexpression (Fig. 5b). Therefore, the residual Cdk1 activity in Sic1 overexpressing cells is insufficient to activate resection of DNA ends, but still enough to repress the recruitment of NHEJ proteins at DSBs and NHEJ activity at G_2.

4. Discussion

Both NHEJ and HR are efficient in repairing DSBs, but they produce products with distinctly different mutational potentials. Proper choice of repair mechanism for a given DNA lesion will minimize cellular mutational load and is pivotal to maintain genome integrity. Previously, evidence indicated that HR efficiency fluctuates throughout cell cycle, with the key recombination step(s) being under control of the Cdk1 kinase [11,12,16]. Here, we confirmed the effect of cell cycle on HR and demonstrated an effect on NHEJ as well; NHEJ is most active at G_1 but substantially attenuated at G_2. Repression of NHEJ at G_2 depends on Cdk1-dependent modulation of end processing and on Yku and Lif1 recruitment to DNA breaks. Importantly, elimination of HR was not sufficient to increase NHEJ at G_2. We propose that regulating the affinity of Yku and Dnl4/Lif1 proteins to DNA breaks defines a novel way to enforce cellular repair pathway choice during the cell cycle in budding yeast.

Being the main engine for cell cycling, Cdk1 is an ideal molecule to coordinate the choice of DSB repair pathway. This insures the availability of the most desirable recombination template: an identical sister-chromatid. HR and NHEJ are oppositely affected by Cdk1 activity: Cdk1 activates HR, but represses NHEJ. Cdk1 controls cell cycle dependent repair pathway choice at multiple steps. First, Cdk1 activates nucleolytic processing of DNA ends to produce recombinogenic ssDNA, an essential intermediate for HR that is also inhibitory to NHEJ [11,12]. Secondly, Cdk1 is important for a later step in homologous recombination, after strand invasion and before the initiation of new DNA synthesis [11]. Thirdly, expression/post-translational modifications of Sae2/CtIP also fluctuate throughout cell cycle [22], with the protein being shown recently to be a target of Cdk1 in yeast and mammals. In this study we demonstrated that Cdk1 additionally represses NHEJ at G_2 by inhibiting the recruitment of at least two NHEJ factors, Yku and Lif1, to DNA breaks.

Since the recruitment of core NHEJ proteins is inhibited by Cdk1 activity, an open question is whether Cdk1 directly phosphorylates one or more core NHEJ proteins to modulate binding affinity to DNA breaks. Direct phosphorylation of Ku by Cdk1 is highly attractive as Ku70 was previously identified as a target of cyclin dependent kinase in mouse [42]. However, removal of all the putative Cdk1 phosphorylation sites (S/TP) on Yku70 and 3 out of 4 sites on Yku80 failed to elicit any DSB repair phenotype, suggesting that the regulatory role Cdk1 on Yku is likely indirect (Y.Z. and S.L., unpublished observations). Since Dnl4/Lif1 can positively influence the binding of Ku at DSBs, it will be interesting to test whether Dnl4/Lif1 are the relevant substrate(s) of Cdk1 [28]. Alternatively, other post-translational modification of Ku (e.g. sumoylation)[43] might be required for DNA binding and regulated by Cdk1.

In this study, we provide compelling evidence that NHEJ is actively suppressed at G_2 by direct inhibition of NHEJ factor binding to DSBs. However, it still remains possible that commitment to NHEJ is compromised at this cell cycle stage due to elevated end resection rather than to direct modulation of NHEJ factors. For instance, Sic1 might cause a gross resection rate decline by selectively repressing certain steps of end resection, but fail to inhibit step(s) germane to NHEJ suppression. As evidence emerges that resection is a multi-step process governed by functionally overlapping nucleases and the associated protein complexes, it remains formally possible that Sic1 may inhibit distinct resection step(s) but leave others intact. In this regard, we would like to emphasize that the earliest step of resection is effectively inhibited by Sic1 overexpression in the PCR-based resection assay.

Interestingly, Clerici et al. previously reported no apparent difference in the amount of *MYC* epitope tagged Yku proteins at an HO-induced DSB at G_1 versus G_2 by ChIP assays [44]. Although the reason for this disparity is unknown, some of the difference can be explained by the non-identical behavior between *MYC*-tagged Yku70 and an untagged Yku protein complex in ChIP assays. In fact, very different outcomes were reported between ChIP assays using an epitope tagged version of Yku and those using a polyclonal antibody raised against Yku to examine association of Yku with telomeres and the *HM* loci [45].

The cell cycle dependent fluctuation of repair pathway efficiency likely reflects evolutionary pressure to preferentially use recombination for DSB repair when sister-chromatids are available, as recombination between sister-chromatids will most faithfully restore the integrity of broken chromosomes. Indeed, DSB repair mutants in other organisms exhibit radiation sensitivity profiles highly suggestive of cell cycle influence [13-15]. We speculate that a similar strategy is applied to DSB repair in mammalian cells where NHEJ plays a more prominent role in repairing DNA breaks.

In summary, the results described here have uncovered a novel role of *Saccharomyces cerevisiae* Cdk1 in the suppression of NHEJ at G_2. Importantly, this regulation minimizes genetic mutation during repair. Our results shed light on how the two major pathways of DSB repair are coordinated during the cell cycle and reveal additional details of the molecular basis of this control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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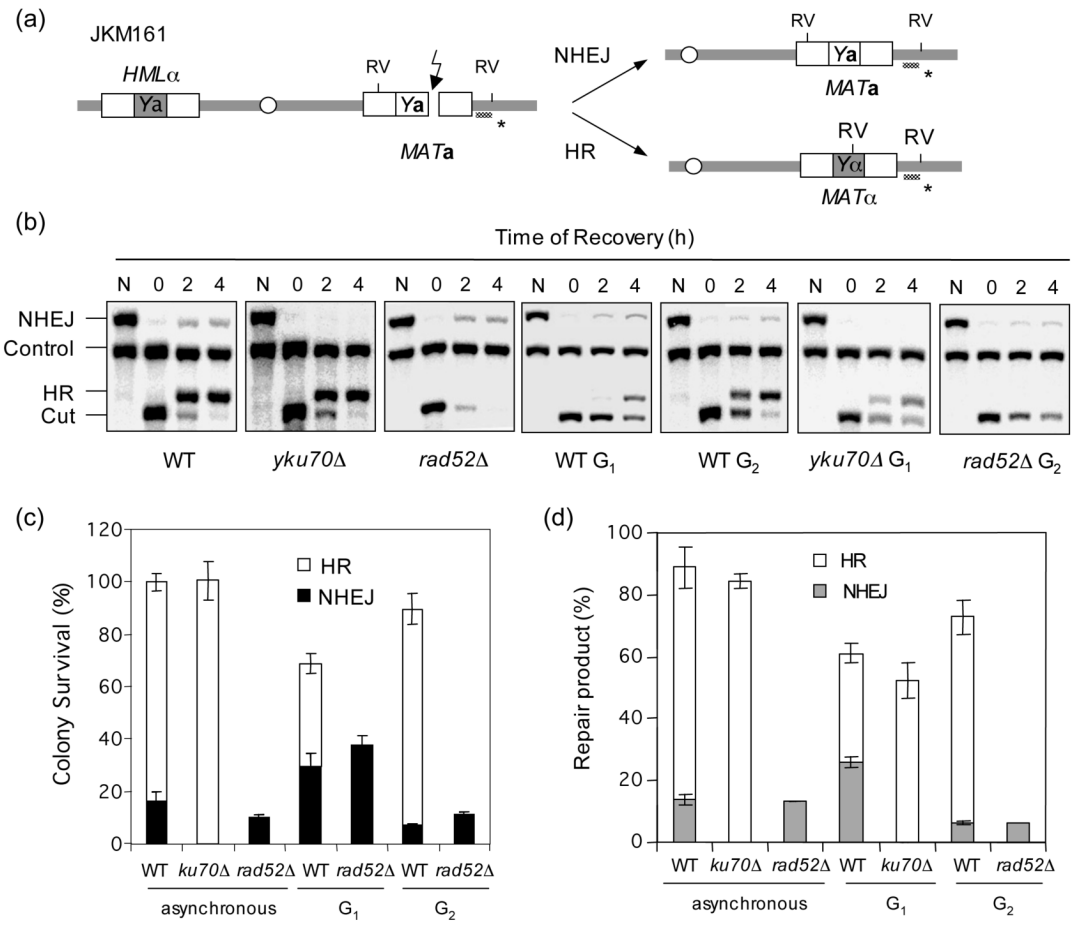

Figure 1.

NHEJ is repressed at G_2. (a) Schematic diagram showing relevant genomic structure of JKM161. The location of a *MAT* specific probe (*) and the restriction endonuclease cleavage sites (EcoRV: RV) used for Southern blot analysis to detect repair product formation are indicated. (b) Southern blot analysis of the repair product formation. N represents no galactose. (c) Plot demonstrating percent colony survival by HR and NHEJ. (d) Plot demonstrating percentage of repair products by HR and NHEJ. Percent repair product was calculated by dividing the repair product signal (HR or NHEJ in (b)) with the signal of the HIS3 control (control) after 4 h of recovery in the glucose containing medium. Data represents the mean ± s.d. of three or more independent experiments.

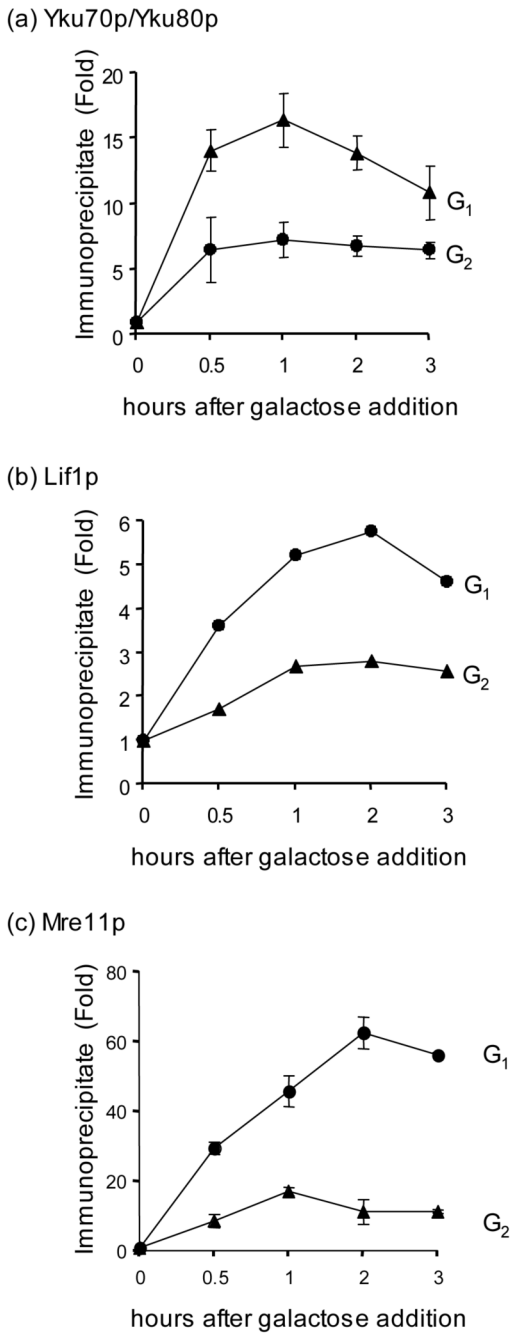

Figure 2.

The recruitment of NHEJ proteins at DSBs is reduced at G_2. Kinetics of Yku (a), Lif1 (b), and Mre11 (c) recruitment to the HO induced DSB in G_1 and G_2 were determined by ChIP assays. Fold immunoprecipitate represents the ratio of the Yku, Lif1, or Mre11 IP PCR signal before and after HO induction, normalized by the PCR signal of the PRE1 control. Data represent the mean ± s.d. of three or more independent experiments.

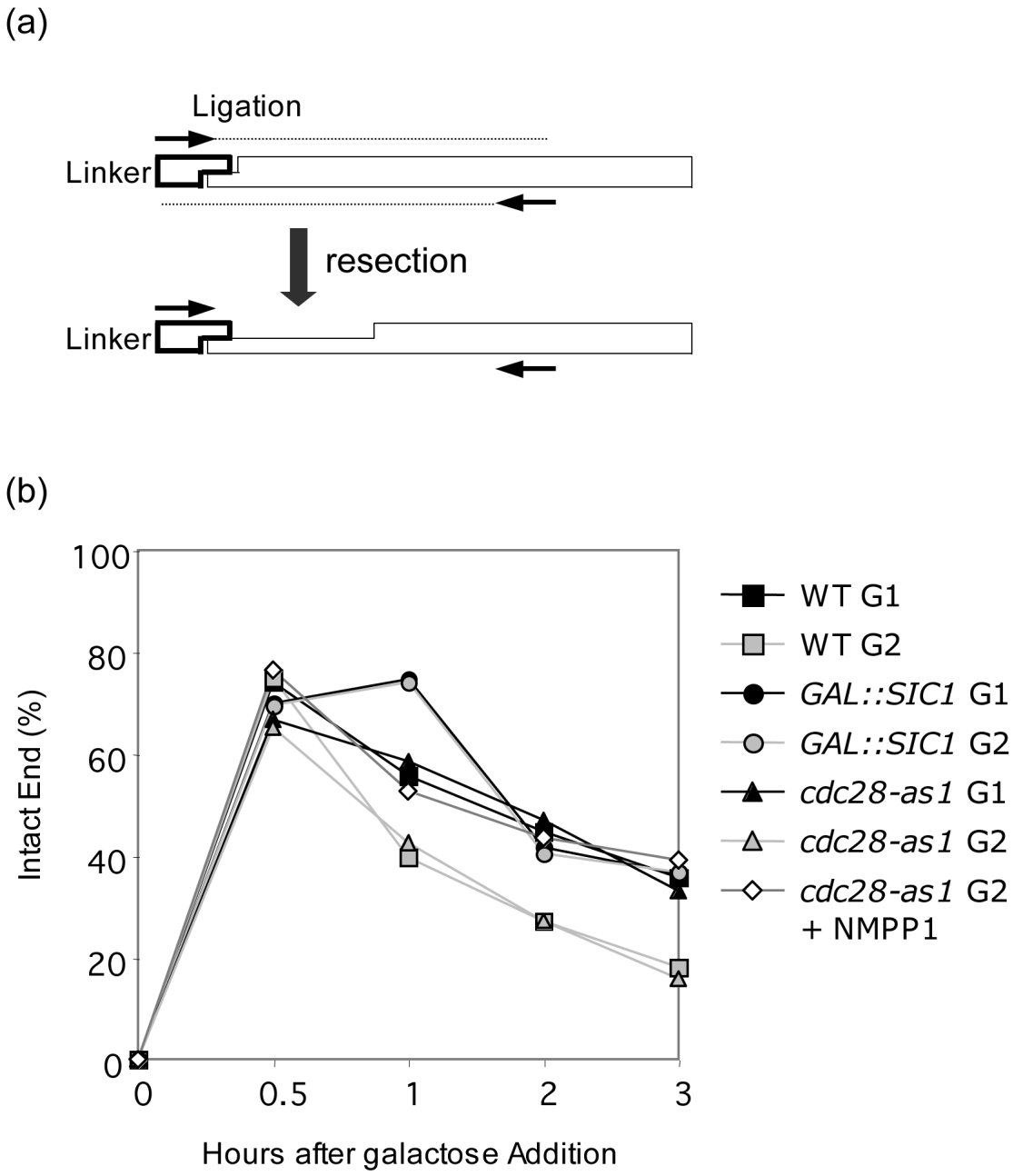

Figure 3.

Cdk1 activity suppresses end resection. (a) Scheme of the ligation-mediated PCR (LM-PCR). The adaptor, fully complementary to the 4 nucleotide 3′ overhang generated by HO induced cleavage, is ligated to genomic DNA. The two primers (arrows) anneal to the adaptor and 0.4 kb distal to HO cut site and will only amplify DNA ends with intact, unresected 5′ chromosomal ends. Even a single base pair of resection will prevent the ligation and leave no signal in the PCR reaction. (b) Percentage of intact DNA ends remaining at HO cleavage site at the indicated time after HO induction. Genomic DNA isolated at each time interval after HO expression was subjected to LM-PCR as described in the Materials and Methods. Percent intact ends is shown as the ratio of the PCR signal from each ligation reaction sample before and after HO induction, normalized by the PCR signal of the PRE1 control. To calculate percent intact ends, the ratio

of the LM-PCR signal from Lev473 strain carrying HO recognition sequences modified to a *Bst*XI site, whose digestion generates the same 4 nucleotide 3′ overhang as HO cleavage, normalized by the PCR signal of the PRE1 control after *Bst*XI cleavage, was set to 100% [25].

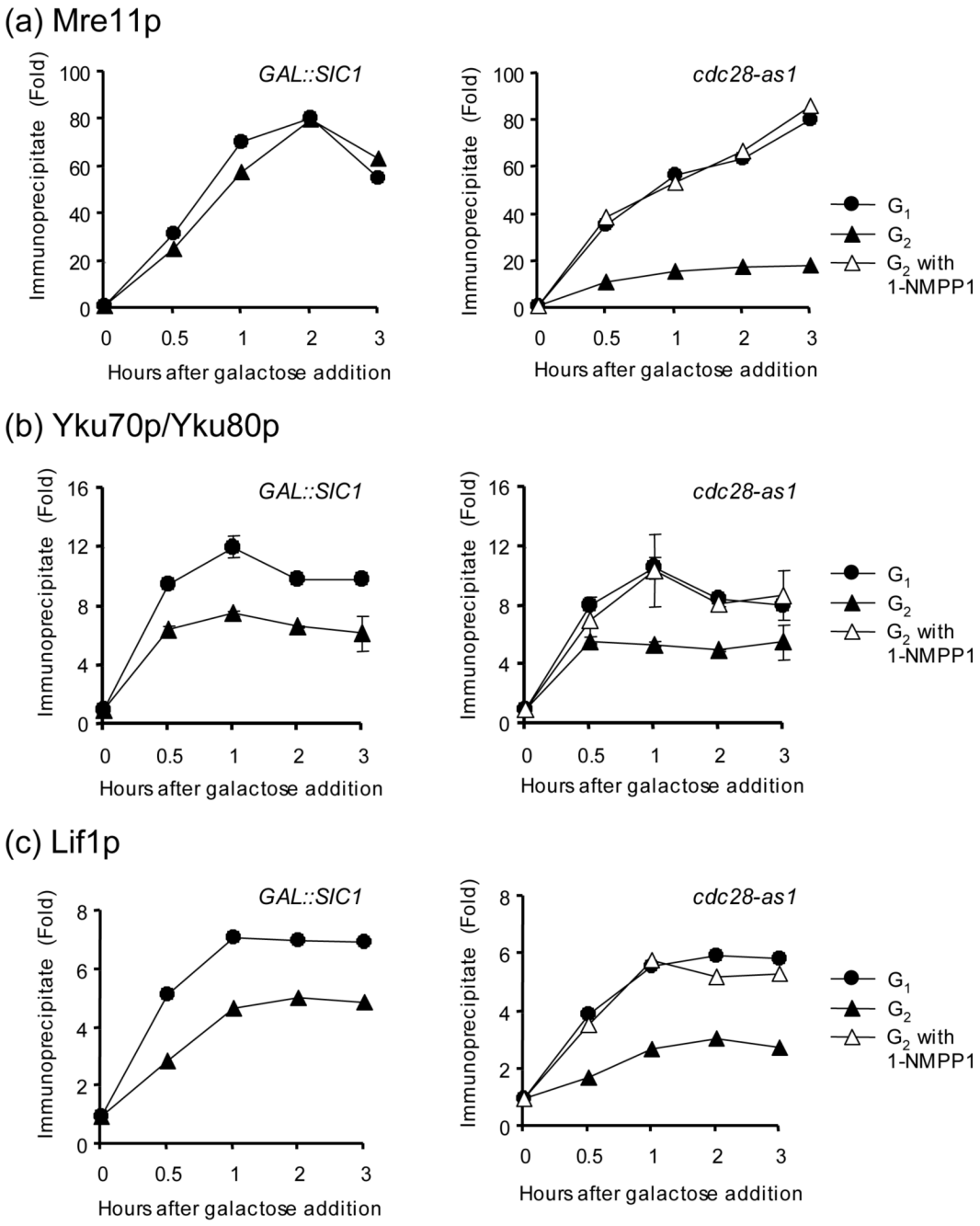

Figure 4.

The recruitment of NHEJ proteins at DSBs is suppressed by the Cdk1 activity but not by end resection. Kinetics of Mre11 (a), Yku (b), and Lif1 (c) recruitment to the HO induced DSB in G_1 and G_2 from strains expressing galactose inducible Sic1 (left panel) or harboring *cdc28-as1* allele supplemented with 1-NMPP1 inhibitor (right panel) were determined by ChIP assays. Fold immunoprecipitate was calculated as described in the legend for Fig. 2. Data represent the mean ± s.d. of two or more independent experiments.

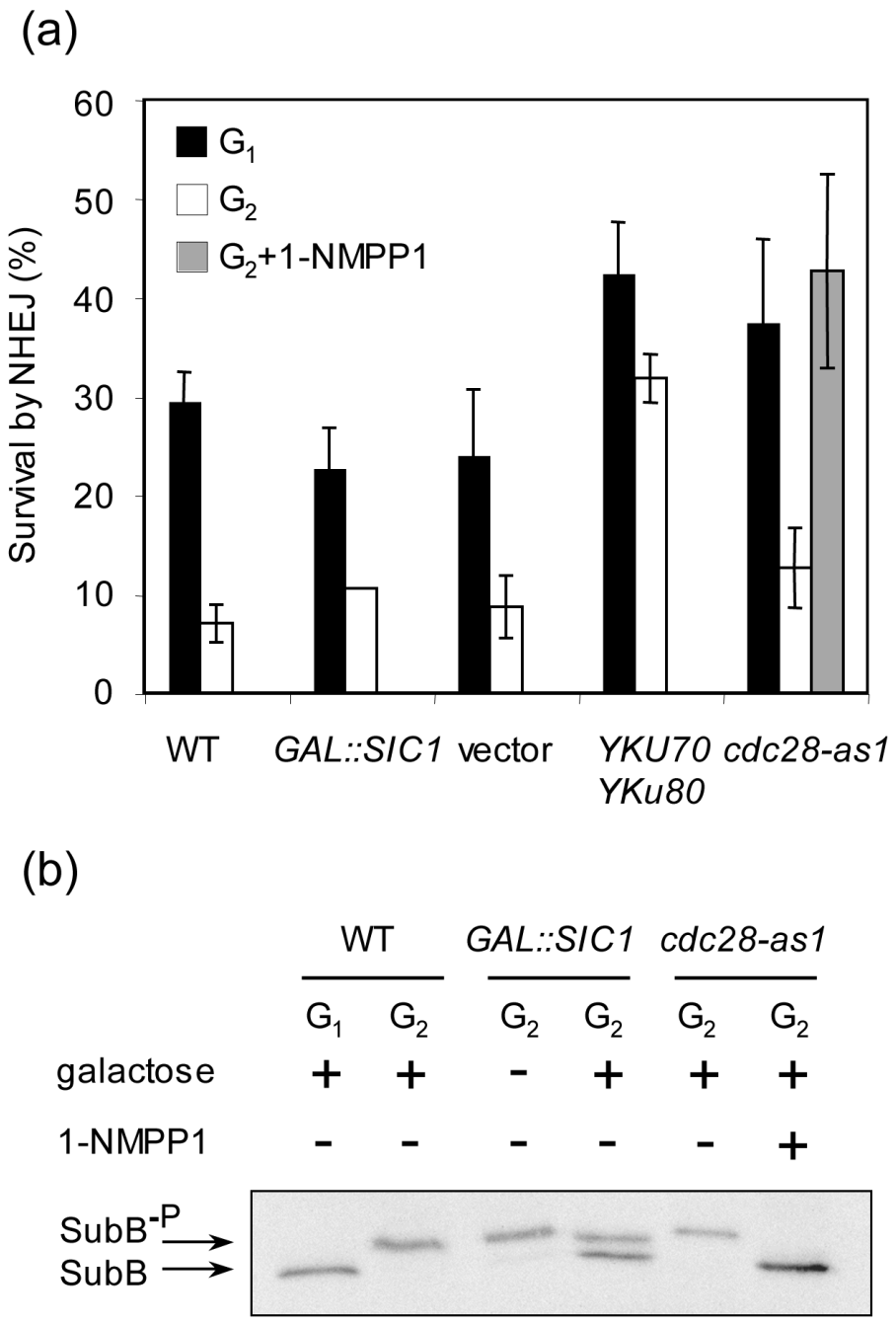

Figure 5.

NHEJ is repressed by Cdk1 activity. (a) Effect of Cdk1 inactivation on colony survival by NHEJ of an HO-induced DNA break. Cultures were induced to express HO for 1 h and plated onto YEPD medium, which shuts off HO expression. Percent survival by NHEJ is shown as the number of **a** type colonies growing onto the YEPD plates after 1 h of HO induction, normalized by the number of colonies growing before HO induction. Data represent the mean ± s.d. of three or more independent experiments. (b) Cdk1 activity was measured by the phosphorylation of the B subunit of DNA polymerase-alpha, a marker for Cdc28/Clb activity [11].

Table 1

Genotypes of strains used in the study

Strain	*Genotype*	*Reference*
JKM161	*hoΔ MAT**a**HMLalpha hmrΔ::ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO*	[28,30]
JKM179	*hoΔ MAT**a**hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO*	[28]
CY6012a	JKM179 *GAL::SIC1-myc*	[11]
tGI561	JKM179 *cdc28-as1*	[11]
YZY46	JKM161 *yku70Δ::URA3*	[28]
YZY51	JKM161 *rad52Δ::TRP1*	This work
YZY105	JKM179 *LIF1-MYC::KAN*	[28]
YZY193	JKM179 *cdc28-as1 LIF1-MYC::KAN*	This work
YZY194	JKM179 *GAL::SIC1-myc LIF1-MYC::KAN*	This work
SLY205	JKM179 pKu70, pKu80	This work