Mutations In RAD27 Define A Potential Link Between $G_1$ Cyclins And DNA Replication

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Mutations in RAD27 Define a Potential Link between G1 Cyclins and DNA Replication

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The yeast *Saccharomyces cerevisiae* has three G1 cyclin (*CLN*) genes with overlapping functions. To analyze the functions of the various *CLN* genes, we examined mutations that result in lethality in conjunction with loss of *cln1* and *cln2*. We have isolated alleles of *RAD27/ERC11/YKL510*, the yeast homolog of the gene encoding flap endonuclease 1, *FEN-1*. In *cln1 cln2 rad27/erc11* cells arrest in S phase; this cell cycle arrest is suppressed by the expression of *CLN1* or *CLN2* but not by that of *CLN3* or the hyperactive *CLN3-2*, *rad27/erc11* mutants are also defective in DNA damage repair, as determined by their increased sensitivity to a DNA-damaging agent, increased mitotic recombination rates, and increased spontaneous mutation rates. Unlike the block in cell cycle progression, these phenotypes are not suppressed by *CLN1* or *CLN2*. *CLN1* and *CLN2* may activate an *RAD27/ERC11*-independent pathway specific for DNA synthesis that *CLN3* is incapable of activating. Alternatively, *CLN1* and *CLN2* may be capable of overriding a checkpoint response which otherwise causes *cln1 cln2 rad27/erc11* cells to arrest. These results imply that *CLN1* and *CLN2* have a role in the regulation of DNA replication. Consistent with this, *GAL-CLN1* expression in checkpoint-deficient, *mecl-1* mutant cells results in both cell death and increased chromosome loss among survivors, suggesting that *CLN1* overexpression either activates defective DNA replication or leads to insensitivity to DNA damage.

In *Saccharomyces cerevisiae*, the major control point in the cell cycle is the G1-to-S transition known as Start. After passage through Start, cells begin a division program that includes bud emergence, DNA synthesis, and microtubule-organizing center duplication (42). Like many cell cycle transitions, passage through Start is controlled by a cyclin-dependent kinase (Cdc28)–cyclin complex (reviewed in reference 38).

Any one of the three G1 cyclins, *CLN1*, *CLN2*, or *CLN3*, is sufficient for transition through Start (9, 44). However, it is likely that *CLN3*’s role in Start may be qualitatively different from that of *CLN1* and *CLN2*. This is based on a variety of observations, including structural homology, regulation of expression, in vivo activities, and in vitro kinase activities (5, 12, 21, 25, 39, 57, 63). From these data, it has been suggested that the substrates of Cln3 may be different from the substrates of Cln1 and Cln2 and that the primary function of Cln3 might be to activate the transcription of other genes, including *CLN1* and *CLN2* (57).

After progression through Start, cells are committed to DNA replication. The mechanistic details by which the Cdc28-Cln kinase commits cells to replication are not yet well understood. One likely role for the Cdc28-Cln protein kinase is in the activation of the transcription factor, MBF (3, 57). MBF can then induce the transcription of a number of genes required for DNA replication (reviewed in reference 35). The B-type cyclin genes, *CLB5* and *CLB6*, are expressed at about the time of Start and promote transit through S phase (17, 51, 57). The Cdc28-Cln kinase may affect Cdc28-Clb5,6 kinase activity both by activating the transcription of *CLB5,6* (57) and by leading to the degradation of the *CLB*-specific inhibitor, p40sec1 (50).

In addition to having a role as an activator of DNA synthesis after Start, the Cdc28 kinase is a likely target for the inhibition of cell cycle progression by proteins involved in checkpoint pathways. A checkpoint ensures that cells have completed an early event with sufficient fidelity to proceed to a later event (24, 59). If an upstream event has not been completed, the cell cycle must be arrested to allow completion or repair of the incompletely assembled structure. Both DNA damage and incomplete DNA replication result in checkpoint-mediated cell cycle arrest (59, 61).

In this work, we describe mutations in *RAD27/ERC11/YKL510*, the yeast homolog of the gene encoding FEN-1, a mammalian structure-specific endonuclease (23). Mutant alleles of *rad27/erc11* result in defects associated with DNA replication and repair. In the absence of both *CLN1* and *CLN2*, strains with *rad27/erc11* point mutations are inviable at 38°C and arrest as large-budded cells; expression of *CLN1* or *CLN2* suppresses the inviability and Cdc28 arrest in the mutants, apparently without significantly affecting the levels of DNA damage. *CLN1* and *CLN2* may either activate another pathway for DNA synthesis without affecting DNA repair or, alternatively, override a checkpoint block caused by the DNA damage found in *erc11* mutant strains. Consistent with *CLN1* altering regulation of DNA replication, *CLN1* overexpression is lethal in cells defective for *MEC1*, a DNA damage checkpoint component.

MATERIALS AND METHODS

Strains and media. Media and genetic methods were as described elsewhere (4, 47). All yeast strains were isogenic with BF264-15D (trpl-1a leu2-3,112 lacI2 his3). Mutant *cln1*, *cln2*, and *cln3* alleles, *CDC28*:HA, and the *GAL-CLN1* and *GAL-CLB5* cassettes have been described previously (10, 11, 16, 44). A derivative of the LEU2::GAL1::CLN1 cassette in which LEU2 was inactivated with *URA3* was constructed by transforming a *LEU2::GAL1::CLN1* strain with an HpaI-Sall fragment of pLU23 (9a). pLU23 contains the *LEU2* gene disrupted by introduction of the *URA3* cassette from *JAX5* (obtained from S. Hughes) into the EcoRV site of *LEU2*. This converted *Lau2::LEU2::GAL1::CLN1* to *Lau2::URA3::GAL1::CLN1*.

Disruption of *RAD9* was accomplished by integrating pTW032 (rad9::TRP1) (60) into strain 1242-8B. Disruption of *RAD2* was accomplished by integrating *UAS5* (rad2::URA3) (obtained from L. Prakash) into strain 2507-5B. In both cases, transformants were scored for radiation sensitivity basically as described...
elsewhere (60). Other strains containing the rad9 or rad11 disruption were generated by crossing to the original transformants. The mec1-1 allele (62) was backcrossed five times to BF264-15D strains; multiple strains were examined for all phenotypes.

The isolation of erc11-1 and erc11-2 has been described previously (5). Because of the bookkeeping error (4a), strains containing these alleles were incorrectly reported to have a non-Cdc-14 arrest phenotype (5), whereas in fact they had not been tested. We report here the first characterization of the erc11 arrest; both erc11 alleles result in first-cycle Cdc arrest.

Cloning of RAD27/ERC11/KYL510. Strain 1983-5B (cln1 cln2 CLN3 GAL-CLN1 erc11-2) was transformed with YCp50- and CE101-based genomic libraries (8, 17), and transformants were screened for their ability to grow at 38°C on YPD plates (47). Complementing plasmids were recovered by transformation into Escherichia coli, analyzed by restriction digestion and Southern blotting, and mapped by hybridization to a chromoblot and lambda phage grid.

To demonstrate that we had cloned ERC11, plasmid LB46 was digested with BamHI and used to transform strain 2507-5D (MATa cln1 cln2 CLN3 ura3). Integration was confirmed by Southern blotting, two independent transformants were crossed to 2522-23B (MATa cln1 cln2 CLN3 leu2-GAL1-CLN1/LEU2 ura3 erc11-2), and tetrad analysis was performed.

Plasmid constructions. Plasmid pB16, recovered from the YCP50-based genomic library, was digested with BamHI, treated with Klenow fragment, and religated to form plasmid pLB50.

A 3.8-kb EcoRI fragment from the YCP50-based genomic library plasmid pLB17 was subcloned into pRS316 (54) to form pLB44. A 4.4-kb EcoRI fragment from LB16 was subcloned into RS316 to form pLB39. pLB39 was digested with XhoI and religated, which deleted a 3.5-kb fragment and resulted in LB37. The EcoRI/HindIII fragment from LB37 was cloned into pLB36, pLB44 was digested with Clal and religated to form pLB52 and pLB53. In pLB44, there are two ClaI sites present in the genomic DNA insert, as well as one site in the vector sequences; pLB53 contains a deletion of all the genomic DNA to the second ClaI site. pLB52 is most probably the result of a partial digest and contains a deletion of the genomic DNA to the first ClaI site.

A 2.8-kb BglII fragment from pLB17 was subcloned into pRS306 and pRS316 digested with BamHI, resulting in pLB46 and pLB48. pLB46 was cut with XhoI and religated, and pLB48 was treated with Klenow fragment, and religated to form the Clal site within the polylinker sequences. The resulting plasmid was digested with Clal and BamHI to delete a portion of the RAD27/ERC11 coding sequence (from bp −60 to +756 relative to the ATG start codon), which was replaced by a TRPII/Kanr cassette from pJA52 (obtained from S. Elledge) to form pLB46. pLB48 was digested with Clal and BamHI and used at a dilution of 1:100, and secondary antibody was used at a dilution of 1:1,000.

Mutation rate analysis, mitotic recombination and chromosome loss assays, and sensitivity to MMS. Rate of mutation to Can was calculated by the Lea-Coulson method of the median (33). Single colonies from YCP50 plates were grown in YPD or YPGal medium. Nine independent cultures of each genotype were analyzed in the first experiment, and 15 independent cultures of each genotype were analyzed in the second. To analyze the amount of chromosome loss and mitotic recombination in rad27/erc11 mutant strains, ERC11, erc11-1, and erc11-1::TRPII diploids (len1::LEU2;GAL1-CLN1/len2::URA3;GAL1-CLN1; note that len2 and MAT are on opposite arms of chromosome III) were purified and a single colony was used to inoculate SC-Gal-Ura-Leu medium (47). Cultures were grown at 30°C for 12 h to allow cells to enter stationary phase, diluted in YPGal or YPD, and grown for 12 h. Approximately 5 × 10^5 cells were mixed in an equal number of W303a or W303b cells and plated on MMS plates for 2 min at 38°C. After 2 min, cells were spotted on SC-Dex-Ada-His to determine number of viable cells and mating events were scored on SC-Dex-Ada-His and SC-Mec1-1/Ada-His plates. Cells were scored as those which resulted in loss of both MAT and the linked URA3 or LEU2 marker.

For the mec1-1 mutant strains, diploids (MEC1 or mec1-1 and len1::LEU2;GAL1-CLN1/len2::URA3;GAL1-CLN1) were purified and a single colony was used to inoculate SC-Dex-Ura-Leu medium. Cultures were grown to early log phase and diluted 1:100 in YPRAf and grown for 12 h. These cultures were then diluted in YPGA or YPD and grown for 12 h. Mating efficiencies were performed as described above. To analyze the sensitivity of strains to MMS, exponential-phase cultures were grown in YPGal and spread on YPD or YPGal plates. Sterile paper discs (diameter, 0.635 cm, no. 740-E; Schleicher & Schuell, Keene, N.H.) containing 0.1 µl of MMS was placed on the surface. Plates were incubated at 30°C for 2 days.

Plating efficiency assays. Tenfold serial dilutions in water were made from fresh stationary-phase cultures, and 5 µl from each dilution was plated. Plates were incubated for 2 days.

RESULTS

Isolation and characterization of mutant alleles of RAD27/ERC11/KYL510. To identify potential targets of the Cdc28-Cln kinase required for cell cycle progression, we have analyzed mutations that result in lethality in a cln1 cln2 CLN3 background at 38°C and are suppressed by expression of CLN1 (see

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These mutations cause an elevated requirement for **CLN** function (Erc²) because **CLN3** is usually sufficient to promote transition through the cell cycle. The screen for **erc** alleles was performed with a **cln1 cln2 CLN3** strain containing **GAL-CLN1** on a **CEN**-based plasmid (5).

The Erc² phenotype (i.e., viability on galactose and inviability on glucose) was determined to be due specifically to the presence of the **GAL-CLN1** construct by both plasmid loss experiments and backcrossing the putative mutants. Only those mutants which required **GAL-CLN1** for their viability on galactose were retained for further analysis (5), ensuring that suppression on galactose was due to the expression of **CLN1**.

Analysis of strains carrying either of the two **erc11** alleles isolated in the screen demonstrated that they are able to grow on glucose-containing media when they are transformed with plasmids containing **CLN1** or **CLN2** under the control of their endogenous promoters (CEN-based and 2μ-based vectors; see Fig. 6B). Furthermore, we constructed and analyzed strains containing the **erc11-2** allele and an intact chromosomal copy of either **CLN1** or **CLN2**. It is critical to note that these strains are able to grow on glucose- or galactose-containing media (i.e., **CLN1 cln2 cln3 erc11** and **cln1 CLN2 cln3 erc11** strains are viable, while **cln1 cln2 CLN3 erc11** strains are not). This demonstrates that wild-type levels of **CLN1** or **CLN2**, but not of **CLN3**, are sufficient for rescue of the **erc11-2** allele. It also confirms that the rescue of **cln1 cln2 CLN3 GAL-CLN1 erc11** strains on galactose as opposed to glucose is not simply due to the carbon source. Finally, **CLN3-2** (an activated allele of **CLN3**) and **GAL-CLN3** were unable to suppress **erc11-2**. Taken together, these data demonstrate that the inviability of the **erc11** mutants on glucose is not simply due to a change in growth rate, or to an inability to survive on glucose medium, but rather is due to the specific loss of wild-type levels of the **CLN1** or **CLN2** gene product.

Of 28 complementation groups with the Erc⁻ phenotype, only those containing **erc11** exhibited first-cycle, cell division cycle (Cdc⁻) arrest as large-budded cells (55a). **cln1 cln2 erc11** mutant cells with **GAL-CLN1** arrested with this phenotype and with first-cycle kinetics when shifted from galactose to glucose at 38°C (Fig. 1 and 2B) or from glucose at 30°C to glucose at 38°C (data not shown). Furthermore, **erc11-2** strains lacking **GAL-CLN1** also displayed these phenotypes when shifted from galactose to 30°C to galactose at 38°C (a further control for carbon source effects; data not shown). **cln1 cln2 erc11-2** cells arrested with a single nucleus and short spindle under the nonpermissive condition (Fig. 1). The **cln1 cln2 erc11-2** strains gradually lost viability at 38°C, with only 10% viability after 6 h of incubation. We do not know the explanation for the low reversibility of the **erc11** block.

Arrest with the morphology described above is frequently associated with a block in DNA replication (6). To determine if **erc11-2** affects DNA synthesis, we examined the DNA content of mutant **erc11-2** cells by FACS analysis (Fig. 2). **ERC11** strains grown with **CLN1** expressed showed a bimodal distribution of DNA content, with peaks at 1 and 2 N (Fig. 2A). The budding index of the cultures was also consistent with their distribution throughout the cell cycle (Fig. 2B). Upon repression of **CLN1** expression by the addition of glucose, the **cln1 cln2 ERC11** strains showed an increase in the number of un budded, **G1** cells as they delayed in their transit through Start (1 to 2 h after addition of glucose). For the **ERC11 cln1 cln2 CLN3** strain, the number of budded cells then increased and the population redistributed into approximately equal **G1** and **G2** populations (by 4 to 5 h after addition of glucose).
ERC11 cln1 cln2 cln3 strain, cells remained unbudded and accumulated in G1, as expected for a strain devoid of G1 cyclin function. The increase in fluorescent staining in these cells may be due to continuing replication of mitochondrial DNA.

When CLN1 was expressed, the erc11-2 mutant cells were distributed throughout the cell cycle (Fig. 2), although the profiles of the erc11-2 strains were somewhat different from that of the wild type. FACs analysis demonstrated that there were fewer cells in the S and G2 phases of the cell cycle and the budding index showed a corresponding increase in the number of large-budded cells, suggesting a delay in transit through the S and/or G2 phases of the cell cycle. The difference between ERC11 and erc11-2 mutant cells was more pronounced with the erc11-2 cln1 cln2 CLN3 GAL-CLN1 mutant strain than the erc11-2 cln1 cln2 cln3 GAL-CLN1 strain; the presence of CLN3 may decrease the amount of time spent in the G1 portion of the cell cycle and lead to accumulation of S/G2 phase cells.

Upon repression of GAL-CLN1 expression by the addition of glucose, the erc11 strains showed an increase in the number of un budded, G1 cells, similar to what was observed with wild-type strains. However, in contrast to wild-type cells, the erc11-2 cln1 cln2 CLN3 cells appeared to pass only slowly through S phase and failed to redistribute into a discrete G2 peak at 3 to 4 h after repression of CLN1 transcription. This demonstrates that with repression of CLN1, transit through S phase is blocked or significantly delayed in the mutant. The increase in fluorescent staining (possibly due to replication of mitochondrial DNA; see above) makes it difficult to be certain of the exact nuclear DNA content in these cells at later time points. However, comparing the erc11-2 cln1 cln2 CLN3 and erc11-2 cln1 cln2 cln3 strains indicates that in the first cycle following shift to restrictive conditions, erc11-2 cln1 cln2 CLN3 cells synthesized some DNA but may have been unable to complete replication. The percentage of budded cells continued to increase in the erc11-2 cln1 cln2 CLN3 culture, and by 6 h after the shift, about 85% of the cells were arrested with large buds.

To confirm that erc11-2 cells arrested in S phase, we used pulsed-field gel electrophoresis since chromosomes isolated from cells arrested in S phase fail to band properly on these gels (26). Most probably this is due to the presence of replication bubbles, forks, or flaps in the DNA which affect its migration ability. As expected, DNA isolated from cells blocked in S phase by a temperature-sensitive DNA ligase inhibitor, nocodazole, demonstrated the characteristic pattern of chromosome bands (12th lane). Under permissive conditions, DNA isolated from an erc11-2 mutant strain showed a chromosome-banding pattern similar to that of the parental ERC11 strain (Fig. 3, third through fifth lanes compared with seventh through ninth lanes). In contrast, under nonpermissive conditions, i.e., in the absence of CLN1 expression at 38°C, the DNA isolated from the erc11-2 mutant fails to band (Fig. 3, sixth lane). Taken together with the FACs data, this suggests that the erc11-2 mutant cells arrest in S phase.

Analysis of mutant erc11-1 strains yielded results similar to those for erc11-2 strains in the pulsed-field gel electrophoresis assay. Minor alterations in the mobility of specific chromosomes seen for the erc11-2 mutant under permissive conditions were not observed in the erc11-1 mutant.

Cloning and characterization of YKL510/ERC11: homology to FEN-1 and RAD2. We cloned the ERC11 gene by complementation of the erc11-2 phenotype. Nineteen plasmids, containing DNA from four different genomic loci, were isolated. Two loci were identified as CLN1 (three plasmids) and CLN2.
The FEN-1 protein has been demonstrated to have DNA endonuclease and 5′→3′ exonuclease activity. FEN-1 has also most likely been purified as a protein, sometimes called MF-1, required for replication in vitro (18, 27, 56, 58). This factor has been demonstrated to be required for completion of lagging-strand DNA synthesis. In addition to its activities on DNA, it has 5′→3′ RNA exonuclease activity on RNA-DNA hybrids and is likely required for the removal of ribonucleotides at the 5′ end of Okazaki fragments.

**Rad27/ERC11 transcription is cell cycle regulated and induced by DNA damage.** The transcription of many genes involved in DNA synthesis is cell cycle regulated, peaking in late G1, and being dependent upon promoter elements called MluI cell cycle boxes (MCB elements) (reviewed in reference 35). The upstream region of **Rad27/ERC11** contains three **MluI** sites, as well as three elements with a single base pair change. The **Rad27/ERC11** transcript was cell cycle regulated, peaking in late G1, similarly to **Cln2** (Fig. 5A). The cell cycle regulation of transcription was evident by two different methods of cell synchronization. In the experiment shown in Fig. 5A, cells were synchronized by arrest in M phase by incubation of **cdc15-2** mutant cells at the nonpermissive temperature and

![FIG. 4. Localization of **ERC11/YKL510**. The arrow indicates the extent of the **ERC11/YKL510** open reading frame and predicted direction of transcription. Lines indicate the genomic DNA present in plasmids, and X indicates a frameshift mutation. Complementation was scored as the ability of a CEN-based plasmid to complement the temperature-sensitive phenotype of 1983-5B (cln1 cln2 CLN3 GAL-CLN1 erc11-2) on glucose media at 38°C. Restriction enzyme sites are designated as follows: B, BamHI; G, BglII; C, ClaI; R, EcoRI; H, HindIII.

The MluI sites were complemented for the **ERC11** loci (11 plasmids) corresponded to **Rad27**/ **ERC11**/ **YKL510**. The third locus (11 plasmids) were able to suppress the **erc11-2** defect when present on CEN-based plasmids (see above). To identify the **ERC11** plasmid, a genomic fragment from the locus was meiotically mapped by targeted integration of **URA3** followed by tetrad analysis of diploids heterozygous for the **URA3**-marked locus and for **erc11-2**. The second locus contained the wild-type **ERC11** gene, we targeted duplicative integration of a plasmid containing **URA3** and DNA from the putative **ERC11** locus into an **erc11-2** mutant strain, 1983-5B. Sixteen of 16 transformants were complemented for the **Erc**− defect, as evidenced by their ability to grow at 38°C in the absence of **CLN1** expression. Subsequently, cells which had removed the duplication by homologous recombination were selected on 5-fluoro-orotic acid. Of the 36 colonies tested, 31 were **Erc**+, demonstrating that the plasmid contained wild-type **ERC11** DNA capable of repairing the **erc11-2** mutation.

We mapped the **ERC11** gene to the right arm of chromosome XI, and we identified a **BamHI** restriction site within the region required for complementation of the **erc11-2** mutation (Fig. 4). Following bidirectional sequence analysis from this site, we determined that **ERC11** had been sequenced as part of the chromosome XI sequencing project and had been named **YKL510** (GenBank accession no. S93804 [28]). **YKL510** was later renamed **Rad27** on the basis of its homology to **Rad2** and the phenotypic analysis of a null allele (43) after our work had been submitted for publication. In this paper, we refer to the gene as **Rad27/ERC11** to be consistent with our figures and the mutations that we have characterized as **erc11** alleles; however, we recognize that the **Rad27** nomenclature has precedence over the **ERC11** designation.

**Rad27/ERC11** is highly homologous to the mouse flaps endonuclease 1 (FEN-1) gene (60% identical amino acids over the entire predicted open reading frame) (22, 23), as well as to **Schizosaccharomyces pombe** rad2+ (56% identical) and a human rad2+ homolog (58% identical) (37). The products of these genes have been implicated biochemically and genetically to have roles in DNA synthesis and repair. They are members of a larger family which includes **Saccharomyces cerevisiae** **Rad2**, **Schizosaccharomyces pombe** rad13+, and human XP-G, which also have roles in DNA repair (7, 20, 48).

![FIG. 5. Transcriptional regulation of **ERC11** mRNA. (A) **cdc15-2** (K2944) cells were grown at 25°C and then arrested in late M phase by shifting the culture to 36°C for 3 h. Synchronous division was induced by shifting cells back to 25°C, and samples for Northern analysis were taken every 12 min. (B) **cln1 cln2 CLN3 GAL-CLN1** (2507-5B MATa or 2101-13C MATa bar−) cells were grown in glucose at 30°C. To arrest cells with pheromone before the addition of MMS, α-factor was added to 2101-13C cells. MMS was added to a final concentration of 0.05%, and samples were taken at 1-h intervals. Quantification of **ERC11** induction was performed by using a Molecular Dynamics phosphoimager, and mRNA levels were normalized by using **TCM1** as a loading control. □, glucose; ●, glucose plus MMS; □, glucose plus pheromone; ■, glucose plus pheromone plus MMS.
The growth of strains containing the erc11::TRP1 allele, as well as the suppression by GAL-CLN1, was somewhat variable among different isogenic isolates. Perhaps the growth defect of the erc11 disruptant strains, coupled with the increased mutation rate caused by erc11 mutations (see below), results in the accumulation of extragenic suppressor mutations. We compared four erc11::TRP1 GAL-CLN1 strains and four erc11::TRP1 strains. Strains with GAL-CLN1 demonstrated about a 3- to 11-fold increase in plating efficiency on galactose compared with the efficiency on glucose at 30°C and a 10- to 900-fold increase in plating efficiency on galactose compared with that on glucose at 38°C. In contrast, erc11::TRP1 strains lacking GAL-CLN1 showed only a 1- to 2-fold increase in plating efficiency on galactose compared with glucose at 30°C and a 1- to 10-fold increase in plating efficiency on galactose compared with glucose at 38°C. These data demonstrate that suppression of the erc11::TRP1 growth defect by galactose medium is largely due to the presence of the GAL::CLN1 gene (although a minor role for galactose as opposed to glucose but independent of GAL::CLN1 cannot be excluded).

The arrest morphology of erc11::TRP1 cells is similar to the phenotype of cells with the point mutation (data not shown), although the Cdc^+ phenotype is slightly less uniform (generally 70 to 80% large-budded cells in erc11::TRP1 arrested strains compared with 85 to 94% in erc11-2 arrested strains). Furthermore, like the erc11-2 mutation, the null allele also appears to block completion of S phase, as assayed by pulsed-field gel electrophoresis (data not shown).

The more extreme phenotype associated with the erc11 null allele demonstrates that the point mutants isolated in the Erc screen do have some residual activity, which may be increased by CLN1. However, since GAL-CLN1 partially suppresses the phenotype associated with the disruption allele, the mechanism of suppression may not be due solely to an increase in the activity of the Ercl11 mutant protein.

Analysis of cell-cycle-regulated transcription and Cdc28-associated kinase activity in erc11-2 strains. The activity of the G1 cyclins has been demonstrated to be important for the transcription of MCB-regulated genes; the Cdc28-Cln kinase may increase the activity of the MCB-binding protein, MBF (3). The presence of MCB elements in the RAD27/ERC11 promoter, coupled with the incomplete rescue of the erc11 null allele by GAL-CLN1, suggested that suppression of erc11-2 by GAL-CLN1 might be the result of increasing the levels of RAD27/ERC11 transcription. Therefore, we analyzed RAD27/ERC11 mRNA levels in the presence and absence of GAL-CLN1 expression (Fig. 7A). In cln1 cln2 GAL-CLN1 strains that contained CLN3, the levels of RAD27/ERC11 transcript decreased transiently when CLN1 expression was repressed, most likely because of the accumulation of cells in the G1 phase of the cell cycle. Then the levels of RAD27/ERC11 mRNA increased and were similar to the steady-state levels in the presence of GAL-CLN1 expression. This was true for both wild-type and erc11-2 strains. However, when strains were devoid of CLN function upon repression of CLN1, RAD27/ERC11 transcript levels decreased substantially when CLN1 was repressed. Taken together, these data demonstrate that CLN3 activity is sufficient for the transcriptional activation of RAD27/ERC11 and make it unlikely that GAL-CLN1 suppresses erc11-2 by transcriptional activation of RAD27/ERC11.

Although GAL-CLN1 does not appear to suppress erc11-2 by a direct effect on the transcription of RAD27/ERC11, GAL-CLN1 might suppress erc11-2 through an effect on the transcription of other genes. One class of notable candidates are the B-type cyclins, CLB5 and CLB6. CLB5 and CLB6 appear to be MCB-regulated genes, and their activity is important for...
We analyzed both the transcript levels of CLB5 and the activity of the Cdc28 kinase in chn1 cln2 CLN3 strains that contained ERC11 or erc11-2, in the presence and absence of GAL-CLN1 expression. Similar to the situation with ERC11 mRNA, levels of the CLB5 transcript transiently decreased upon repression of GAL-CLN1 in chn1 cln2 CLN3 strains and then increased to levels comparable to those present before CLN1 repression (Fig. 7A). The ERC11 and erc11-2 strains behaved comparably to one another. In chn1 cln2 cln3 GAL-CLN1 strains, the level of CLB5 mRNA decreased more dramatically and remained lower than in the chn1 cln2 CLN3 GAL-CLN1 strains for about 4 h after CLN1 repression. For unknown reasons, CLB5 transcript levels increased at later times after CLN1 repression. This experiment demonstrates that in chn1 cln2 CLN3 cells, CLN3 is capable of activating CLB5 transcription to a level similar to the level found when GAL-CLN1 expression is present, in both ERC11 and erc11-2 strains. Therefore, the inefficient replication of chn1 cln2 CLN3 erc11-2 strains is not due to a defect in CLB5 expression. The transcription levels of CDC9, another MCB-regulated gene, were also comparable in the ERC11 and erc11-2 strains in the presence and absence of GAL-CLN1 expression (Fig. 7A).

When we examined the levels of the H2A transcript, which is not MCB regulated (41), we observed a slight reduction in response to turning off GAL-CLN1. This difference was more pronounced in the ERC11 strain than in the erc11-2 strain. We do not yet know the significance of this difference; CLN1 may have a direct effect on the levels of histone transcription, or this may be the result of a difference in the cell cycle distributions between these strains.

Finally, we also examined the transcription of an SCB-regulated transcript, PCL1 (HCS26) (40, 57). The regulation of the levels of the PCL1 transcript was similar to that of CLB5 and was comparable in the ERC11 and erc11-2 strains, in both the presence and absence of GAL-CLN1 expression (data not shown).

Overall, these measurements of transcript levels indicate that in either erc11-2 or ERC11 strains, the presence of CLN3 is entirely sufficient for activating transcription of both SCB- and MCB-regulated genes. CLN3 is required for this activation, at least for several hours following inactivation of GAL-CLN1 (Fig. 7A). Therefore, it is unlikely that rescue of erc11-2 inviability by CLN1 is due to differential activation of transcription by CLN1 as opposed to CLN3.

Although transcriptional levels of CLB5 are similar in ERC11 and erc11-2 strains in both the presence and absence of CLN1 expression, posttranscriptional effects on the activity of the Cdc28-cyclin kinase could account for the cell cycle arrest of erc11 strains. For example, the inactivation of p40Nci1, the Cdc28-Cib kinase-specific inhibitor, requires Cdc28-Cln kinase function. We analyzed the levels of Cdc28-associated kinase activity in ERC11 and erc11-2 strains (Fig. 7B). Because the Cdc28-Cib kinase activity is much greater than the Cdc28-Cln kinase activity (19), most of the kinase activity detected in this assay can be attributed to the Cdc28-Cib kinase.

In the erc11-2 strains grown in the presence of CLN1 expression, there was a high level of Cdc28-associated kinase activity compared with the level in ERC11 control strains. This is probably due at least in part to the increase in the number of cells in the S and G2 phases of the cell cycle (Fig. 2), when the level of Cdc28-Cib kinase activity is high. After repression of CLN1, the levels of kinase activity in both the ERC11 CLN3 and erc11-2 CLN3 strains were similar to the levels present before inactivation of CLN1. In the cln3 strains, which contained no G1 cyclin activity upon repression of CLN1, the levels of Cdc28-associated kinase activity decreased when
TABLE 1. Spontaneous mutation rates in erc11 mutant cells

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rate of Can(^b) events (10^{-6})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GAL</td>
</tr>
<tr>
<td>ERCl1</td>
<td>0.68</td>
</tr>
<tr>
<td>erc11::TRP1</td>
<td>21</td>
</tr>
<tr>
<td>erc11::TRP1</td>
<td>99</td>
</tr>
<tr>
<td>ERC11</td>
<td>1.1</td>
</tr>
<tr>
<td>erc11::TRP1</td>
<td>62</td>
</tr>
</tbody>
</table>

* Cells were plated on YPGal and SCGal lacking Arg and containing canavanine to determine viable and Can\(^b\) cells. Rates were calculated by the Lea-Coulson method of the median (33) from the analysis of 9 independent cultures for each carbon source for the first experiment and 15 independent cultures for each carbon source for the second experiment. GAL, galactose; DEX, dextrose (glucose).

CLN1 was repressed. Although the Cdc28-associated kinase activity assayed is not due directly to Cdc28-Cln kinase, G1 cyclin activity is required for both CLB transcription and inactivation of p40\(^{cde7}\). Taken together with the transcriptional activation of CLB5, these data demonstrate that CLN3 activity in erc11-2 mutant cells is sufficient for the activation of Cdc28-associated kinase, making it unlikely that the block to cell cycle progression is due to a general failure to activate CLB-dependent kinase activity in the erc11 mutant. We do not know the contribution of specific Clb proteins to the bulk Cdc28-associated kinase we have measured. However, the results of Schwob et al. (50) indicate that the six CLB genes are redundant for activation of DNA replication.

ERC11 is important for repair of DNA damage. Many genes involved in DNA replication also have a role in DNA repair. Furthermore, like defects in DNA synthesis, defects in DNA repair might also lead to cell cycle arrest, due to DNA damage checkpoints. Therefore, we analyzed erc11-2 and erc11::TRP1 strains for phenotypes associated with the repair of DNA damage. Although erc11 mutants are not UV sensitive (see below), they do display phenotypes associated with a defect in DNA repair. Surprisingly, CLN1 expression does not suppress any of these erc11 defects.

We analyzed the spontaneous mutation rate in wild-type and mutant cells by assaying the frequency of canavanine-resistant cells (Table 1). Cells containing either the erc11-2 or erc11::TRP1 allele mutated to Can\(^b\) at a higher frequency than did wild-type cells. Mutant erc11 strains are about 10-fold more sensitive than wild-type cells to the DNA-damaging agent MMS, as indicated by a halo assay (Fig. 8A).

Mutations in erc11 cause a striking increase in the recombination rate. Both erc11-2 and erc11::TRP1 cause an approximately 100-fold increase in the loss of heterozygosity at the MAT locus, as determined by the increase in mating proficiency of diploid cells. This increase is due almost exclusively to recombination, as most events resulting in mating competency were associated with the retention of a marker on the opposite side of the centromere from the MAT locus, as would be expected for recombination (but not for chromosome loss) (Fig. 8B).

We also assayed for DNA damage more directly by testing for the presence of nicks. We analyzed the amount of radioactive nucleotide incorporated by Klenow enzyme into DNA isolated from erc11 mutant and wild-type strains as well as the size of the radiolabelled DNA fragments. Analysis of the radiolabelled products by denaturing alkaline agarose gel electrophoresis demonstrated that DNA isolated from both erc11-2 and erc11 null mutant strains had incorporated more label and was on average, smaller than DNA isolated from wild-type strains (data not shown). This effect was largely independent of CLN1 expression, consistent with the observations above suggesting that erc11 causes defects in DNA synthesis and/or repair that are not suppressed by CLN1.

ERC11 and RAD2 have distinct roles in vivo. RAD27/ERC11 and RAD2 are members of the same gene family by sequence. To test for genetic interactions and functional similarities between them, we transplanted rad2 null mutations into cln1 cln2 CLN3 GAL-CLN1 strains. These null mutations did not result in an Erc\(^c\) phenotype. We crossed erc11::TRP1 and rad2::URA3 haploids and examined the meiotic progeny. Spores containing both mutations were recovered at the expected frequency for unlinked genes. The erc11::TRP1 and erc11::TRP1 rad2::URA3 mutants displayed similar plating efficiencies on glucose and galactose at 30 and 38°C. erc11::TRP1 mutants are
not UV sensitive, and the rad2::URA3 and erc11::TRP1 rad2::URA3 mutants displayed similar sensitivities to UV irradiation (data not shown). These results suggest that although Rad2 and Erc11/Rad27 share significant homology, they may have little functional overlap in vivo.

CLN1, erc11, and cell cycle checkpoints. Many mutations that block DNA replication cause a Cdc25 cell cycle arrest due to checkpoint control: the failure to complete replication activates a regulatory process blocking cell cycle progression (24, 59). The RAD9 and MEC1 genes encode components of this checkpoint pathway (59, 62). To test if the Cdc25 arrest of erc11 cln1 cln2 strains was due to a checkpoint control assaying the completion of DNA replication, we wanted to construct erc11 rad9 and erc11 mecl strains. GAL-CLN1 rad9 erc11-2 strains were inviable on both galactose and glucose at 38°C (data not shown). The cln1 cln2 rad9 erc11-2 mutants failed to arrest with a Cdc25 phenotype in either the presence or absence of CLN1 expression, demonstrating that the arrest morphology of cln1 cln2 erc11-2 mutant cells is dependent upon RAD9. Because the cln1 cln2 rad9 erc11-2 mutants now fail to show Cdc25 arrest, we conclude that Cdc25 arrest in the absence of Cln1 is caused by a checkpoint mechanism assaying the completion of DNA replication. The inviability of erc11-2 rad9 mutants suggests that GAL-CLN1 is dependent upon a RAD9 checkpoint for suppression of the erc11-2 defect. The requirement for RAD9 is also consistent with the high level of Cdc28-associated kinase activity in the erc11-2 strains; the RAD9-dependent G1/M checkpoint arrest or pause is associated with high levels of Cdc28-associated kinase activity (55).

We were unable to analyze the effect of the mecl-1 mutation on erc11 mutants, because mecl-1 ERC11 GAL-CLN1 cells demonstrated a severe growth defect on galactose in comparison with mecl-1 cells without GAL-CLN1 (Fig. 9A). On galactose, mecl-1 ERC11 GAL-CLN1 cells continued cell division, which led to an increase in cell number; however, inviable cells accumulated in the population. After 12 h of growth in galactose, approximately 90% of the cells in the culture were unable to form colonies when plated.

To analyze this effect of GAL-CLN1, we monitored chromosome loss and recombination events in diploids by assaying for the formation of mating cells (Fig. 9B). mecl-1 caused about a 10-fold increase in loss and recombination, even in the absence of GAL::CLN1 expression, from the levels obtained with MEC1 strains. A 12-h pulse of galactose to activate GALL::CLN1 transcription resulted in a 100-fold increase in loss and recombination compared with levels for MEC1 controls. Therefore, the inviability caused by GAL-CLN1 expression may be due to effects on DNA synthesis or chromosome segregation.

DISCUSSION

We describe the isolation and characterization of mutations in RAD27/ERC11/YKL510. Mutations in erc11 result in cell cycle arrest of cln1 cln2 cln3 strains, a phenotype that is suppressed by expression of CLN1 or CLN2. Characterization of the erc11 arrest demonstrates that cells are blocked in the completion of DNA synthesis. First, cells arrest with a large bud, a short spindle, and a single nucleus. This phenotype is identical to that associated with other mutations that affect DNA replication. Second, FACS analysis demonstrates that cells are crippled in their capability to synthesize DNA, as their progression is greatly delayed compared with that of wild-type cells, and they may not actually complete synthesis. Finally, DNA isolated from erc11 mutants is unable to band on CHEF gels, as has been described for mutations and conditions with which cell growth is arrested in S phase.

Mutations in erc11 also cause defects in DNA repair, including an increased spontaneous mutation rate, increased sensitivity to a DNA-damaging agent, and increased recombination.

FIG. 9. (A) Inviability of mecl-1 GAL-CLN1 strains. Spores from a diploid strain heterozygous for mecl-1 and GAL-CLN1 were inoculated onto glucose and galactose media and grown for 2 days. The mecl-1 genotype was assigned to spores on the basis of complementation testing with known mecl-1 mutant strains for hydroxyurea sensitivity. The GAL-CLN1 genotype was assigned on the basis of Leu+ prototrophy; the integrated construct is a LEU2::GAL::CLN1 cassette. Dex, dextrose (glucose); Gal, galactose. (B) Recombination and chromosome loss levels in MEC1 and mecl-1 mutant strains. A quantitative mating assay was used to score for the loss of heterozygosity at the MAT locus in diploids after 12 h of growth in galactose or glucose. Chromosome loss events are those which result in loss of both MAT and a linked marker; recombination events result in loss of heterozygosity at MAT and retention of a linked marker on the opposite side of the centromere. Data shown are from the MATa cross; similar results were found with crosses to a MATa tester. DEX, dextrose (glucose); GAL, galactose; recom, recombination.
Although CLN1 expression suppresses the block in replication caused by erc11-2, it fails to suppress any of the defects associated with DNA damage.

The phenotypes associated with erc11 alleles, as well as the cell-cycle-regulated and damage-inducible transcription of RAD27/ERC11, are in vivo evidence for functions for the protein in both DNA replication and repair. A role in these functions was suggested by the in vitro biochemical activity described for Rad27/Erc11/Ykl510, and its mammalian homolog, Fen-1, as a DNA flap endonuclease (22, 23). It was postulated that Fen-1 activity might be required during DNA repair to cleave a damaged strand of DNA after unwinding and during lagging-strand DNA synthesis for cleavage and ligation of displaced Okazaki fragments. After this manuscript was submitted, Reagan et al. published an article characterizing the phenotypes associated with disruption of Rad27/Erc11/Ykl510 in cln1 cln2 cln3 cells (43). They found that disruption of RAD27/ERC11 caused temperature-sensitive cell inviability with defects associated with a block in the completion of DNA replication. Their analysis and conclusions are consistent with the data presented here demonstrating that RAD27/ERC11 has critical roles in both DNA replication and repair.

Isolation of erc11/Ykl510 in a screen for mutations that require increased G1 cyclin dosage. The isolation of mutant alleles of RAD27/ERC11 synthetically lethal with cln1 cln2 defines a link between Start and DNA replication and/or repair. One interpretation of the isolation of rad27/erc11 in this context is that either the fidelity or the rate of DNA replication is broadly compromised in cln1 cln2 cells and, therefore, that a number of mutations in the DNA replication or repair pathway could cause synthetic lethality with cln1 cln2 (an Erc phenotype). For a number of reasons, we do not favor this hypothesis. First, we have been unable to observe any differences between cln1 cln2 CLN3 cells and cln1 cln2 CLN3 GAL-CLN1 cells in sensitivity to MMS, hydroxyurea, or UV irradiation (55, 57a). Second, the numbers of cells in S phase, as assayed by FACS, are not strikingly different for the two genotypes, suggesting that this is not a kinetic slow point in cln1 cln2 CLN3 cells. Third, deletion of rad2, a gene known to be required for some types of DNA repair, does not cause the Erc- phenotype. Fourth, many genes required for DNA replication have been identified; mutations in most cause Cdc- arrest. RAD27/ERC11 is the only complementation group identified in our screen thus far which results in the budded-cell arrest phenotype characteristic of mutants defective in DNA synthesis. Taking these data together, it appears that if cln1 cln2 cells do make defective DNA, the defects are subtle, specific, and not synergistic with those associated with other DNA-damaging agents; if synthesis is indeed defective, Erc11 and only a few other proteins may be specifically required to repair the defects generated in cln1 cln2 cells.

One possible mechanism of suppression of erc11 by CLN1: activation of Erc11 and/or another pathway. Mutant erc11 alleles result in lethality in cln1 cln2 CLN3 cells. The phenotype of these cells demonstrates that in the absence of CLN1 and CLN2, cells are dependent upon RAD27/ERC11 for progression through S phase. One interpretation is that CLN1 and CLN2 can activate another pathway for DNA replication that is at least partially RAD27/ERC11 independent, while CLN3 is unable to activate this pathway. This does not necessarily imply that RAD27/ERC11 is a substrate of the Cdc28-Clb kinase, only that in the absence of CLN1 and CLN2, cells are dependent upon a pathway within which RAD27/ERC11 is an essential component. The ability of CLN1 and CLN2 to activate a DNA synthesis pathway that CLN3 is unable to activate is consistent with a variety of observations that CLN1 and CLN2 promote cell cycle progression in a way qualitatively different from that of CLN3 (see the introduction).

Previously identified roles for the G1 cyclins in control of DNA synthesis include the activation of transcription of genes required for S phase, including the CLB genes, as well as the activation of Cdc28-Clb kinases, probably by inhibition of p40$^{scf}$ (50, 57). CLN3 appears to be at least as potent an activator of transcription as CLN1 (57; also see above), making it unlikely that CLN1 and CLN2 suppress erc11 by activating the general transcription of genes required for DNA synthesis. In particular, we have tested cln1 cln2 strains (both erc11-2 and ERC11) for induction of RAD27/ERC11 itself, as well as the MCB-controlled genes CDC9 and CLB5 and the SCB-controlled gene PCL1, and have observed no defect in their induction in the absence of CLN1 expression. At most, a modest defect in histone H2A transcription as a result of turning off CLN1 expression was observed. Therefore, we consider it unlikely that the defect in cln1 cln2 erc11-2 strains that is suppressed by CLN1 or CLN2 expression is due to defects in transcriptional induction of other genes. However, it remains possible that CLN1 and CLN2 do suppress erc11 via the activation of the transcription of genes not yet identified.

In addition to being proficient in activating transcription of SCB- and MCB-regulated genes, cln1 cln2 erc11-2 strains contained high levels of Cdc28p-associated histone H1 kinase activity, suggesting that the Sic1p inhibitor of Cdc28-Clb kinase activity was not functioning. These results suggest a direct role for CLN1 and CLN2 in regulation of DNA replication independent of activating Clb protein expression and kinase activity (50).

We have identified two possible components of the hypothetical CLN1- or CLN2-dependent pathway, SEL1 (suppressor of erc11, encoding a potential helicase) and CDC9 (encoding DNA ligase). Increased levels of these proteins can suppress the erc11-2 DNA synthesis defect (57b). Furthermore, deletion of SEL1 and ERC11 is synthetically lethal, even in the presence of CLN1, as would be predicted for the disruption of a component in each of two functionally redundant pathways. Finally, like CLN1 and CLN2, SEL1 and CDC9 can suppress the temperature-sensitive lethality of erc11-2 but are unable to suppress the MMS-sensitive erc11-2 phenotype (associated with defects in DNA repair).

The observation that CLN1 and CLN2 are unable to complement the requirement for RAD27/ERC11 in DNA repair could cast doubt on the idea that CLN1 and CLN2 may activate a parallel pathway that can at least partially bypass the requirement for RAD27/ERC11 function in replicative DNA synthesis. Ad hoc explanations to accommodate this fact could include the following: CLN1 and CLN2 can interact with Erc11 at only a subset of the temporal or spatial domains at which Erc11 functions; RAD27/ERC11 has two different activities in vivo, loss of only one of which (required for DNA synthesis) can be suppressed by an alternative pathway activated by CLN1 and CLN2; and a function activated by CLN1 and CLN2 is present at the time appropriate for its having a role in DNA synthesis but is not expressed at a time appropriate for its having a role in DNA damage repair. However, we lack direct evidence in favor of any of these explanations. An alternative explanation is presented below.

Possible mechanism of suppression of erc11 by CLN1: bypass of a checkpoint arrest. Although CLN1 and CLN2 suppress the inviability and cell cycle arrest caused by erc11-1 and erc11-2 in cln1 cln2 CLN3 cells, they are apparently unable to suppress any other erc11 phenotypes. One interpretation of this surprising result is that cln1 cln2 CLN3 cells arrest because of the defects caused by the erc11 alleles and that CLN1 allows
the cells to progress through the cell cycle by ignoring these defects, not by repairing them. It may be that the erk1/2 damage results in a response which inhibits Cdc28-Cln3 kinase but fails to inhibit Cdc28-Cln1 kinase. This differential inhibition may be the result of quantitative or qualitative differences between the Cdc28-Cln kinases.

If CLN1 and CLN2 function causes the bypass of a checkpoint-mediated arrest, the levels of DNA damage or disrepair generated by reducing erk1 function must not be lethal. Otherwise, bypass of the checkpoint would result in cell death. Apparently the levels of damage caused by erk1 are sublethal only when RAD9 is functional. The requirement for RAD9 is consistent with the presence of DNA damage and demonstrates that some checkpoint response is, in fact, required for suppression. Perhaps CLN1 causes a bypass of one checkpoint in erk1 mutant cells, allowing replicative DNA synthesis, but that then a pause at another checkpoint (RAD9 dependent) is required to allow successful completion of DNA synthesis or some repair before mitosis.

It is also interesting in this context that CLN1 overexpression is lethal in cells defective for the MEC1-dependent DNA damage checkpoint (Fig. 9). Perhaps mec1-1 strains are dependent for viability on the same checkpoint bypassed by CLN1 in the erk1-2 background. This idea could lead to the prediction that mec1-1 might be synthetically lethal with rad9; however, this appears not to be the case (unpublished results). Alternatively, CLN1 overexpression could somehow activate defective or error-prone DNA replication, requiring a pause at a MEC1-dependent checkpoint for repair.

Previous work has indicated that some checkpoints do function through the inhibition of cyclin-dependent kinases and cyclins. In Schizosaccharomyces pombe, inhibitory phosphorylation of cdc2p, the Cdc28p homolog, is required for hydroxurea-induced cell cycle arrest; in the absence of phosphorylation, cells experience mitotic catastrophe (15, 34). Furthermore, in mammalian cells, DNA damage in G1 causes cell cycle arrest and decreased CDK activity. This effect is p53 dependent (29, 32) and is due, at least in part, to inhibition of the activity of cyclin E-cdk2 complexes by the p53-inducible p21WAF1/CIP1 protein and reduction of the levels of cyclins A and D1 (13, 14).

It has recently been demonstrated that in Saccharomyces cerevisiae, RAD9, previously shown to be required for arrest in G1/M, is also required for arrest in the G2/S phase after UV irradiation (52, 53). Perhaps RAD9 affects both Cdc28-Cln and Cdc28-Cln kinase activity. Although RAD9-mediated arrest is characterized by high kinase levels at the G1/M checkpoint (55), the size of complexes containing Cdc28-cyclin from checkpoint-arrested cells is different from that of complexes from nonarrested cells. This demonstrates that there is some effect on the Cdc28-cyclin complex. Another possible connection between the Cdc28 kinase and checkpoint is that a loss-of-function mutation in CKSI, a Cdc28-cyclin binding protein and the budding yeast homolog of p130ERF, can suppress a mutation in the checkpoint gene RAD53 (MEC2/SAD1) (2). Rad53p may inhibit Cdc28 kinase activity or alter kinase specificity by affecting CKSI function. Our observations that GAL-CLN1 dramatically affects the viability of mec1-1 strains may reveal an additional link between checkpoints and cyclin-dependent kinases. High, constitutive levels of CLN2 may either cause insensitivity to DNA damage or result in DNA synthesis that is partially defective.

Suppression of erk1 by activation of a partially erk1-independent pathway and suppression by bypass of a checkpoint arrest are not mutually exclusive hypotheses. CLN1 and CLN2 may have multiple effects on the regulation of DNA synthesis.

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