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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)  
In order to identify proteins that play a role in telomeric processing, I took two approaches. The first was a candidate gene approach, and to this end, I directly tested a set of proteins for their involvement in processing the chromosome termini when end protection mechanisms are compromised. Secondly, I designed and carried out a large-scale genetic screen. The screen was based on the assumption that loss of processing at the telomere would make the essential protective function of Cdc13 dispensable. To this end, I screened for genes that, when mutated, resulted in the ability to bypass the lethality associated with loss of Cdc13 function. Unexpectedly, a large number of mutants were isolated from the screen, representing multiple disrupted genes, indicating that many different genes/pathways can contribute to telomeric processing. Many of the genes characterized have telomere phenotypes in otherwise wild-type backgrounds, supporting a potential role in telomere metabolism and maintenance. One class of mutants appears to have been isolated owing to the ability of its members to enhance a previously described recombination pathway for telomere maintenance. Further analysis is critical to understanding whether these proteins play a direct role at the chromosome termini, and if so, by what mechanism they do so.

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## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	10
Reportable Outcomes.....	10
Conclusions.....	10
References.....	12
Figure 1.....	14
Table 1.....	16

## Introduction

Following conventional replication, the chromosome terminus generated as a product of leading strand synthesis will not have the 3' G-rich overhang thought necessary for proper telomeric replication. If downstream events required to process this end are absent or mutated, this terminus will present two problems for the cell. First, it will potentially be recognized as a double strand break and therefore may be susceptible to processes that occur at broken ends, such as fusion and/or degradation. Second, in vitro, telomerase cannot act on a terminus lacking a 3' G-rich overhang, and thus this end should not be a substrate for elongation. Cdc13 has emerged as a key component of the solution to both of these problems, and it acts as such by binding to the telomere and recruiting various complexes needed for telomere function (see Lustig, 2001 for review). However, this model of telomere function and replication predicts a critical event upstream of Cdc13 binding. The protein binds only single-strand G-rich telomeric DNA, implying that such a substrate must be generated prior to recruitment of protein complexes to the telomere.

Current models employ an as-yet unidentified 5'-3' processing activity that occurs normally at the end produced by leading strand synthesis, producing the G-tails normally observed in S-phase, and presumably uncovering the substrate for Cdc13 binding. It has been proposed that in the absence of Cdc13 function, this normal processing activity proceeds unchecked and the cell eventually sustains significant damage and enters a Rad-9 dependent arrested state. This is supported by the observation that there is extensive G-rich single-strandedness at the telomere in cells compromised for Cdc13 function, and the loss of the C-strand proceeds in a telomere to centromere manner (Garvik et al., 1995; Lydall and Weinert, 1995; Booth et al., 2001). This model makes a clear prediction: if the essential function of Cdc13 is to protect the chromosome from unregulated processing, a cell should be able to survive in the absence of Cdc13 if that processing activity is absent.

## Body

*My research efforts the past year have been almost entirely focused on addressing Task #5 from my revised statement of work (see attached). Tasks 1 and 2 are complete, and tasks 3 and 4 were not pursued during the time period in question. Therefore, the body of this report will detail the extensive progress made on task 5.*

### Candidate gene approach towards identification of end processing activities

In my attempts to achieve the goals of this task, I first took a candidate approach to identification of proteins that, when deleted, can promote viability in the absence of end protection. This approach was based on the model that abrogation of telomeric processing would render end protection mechanisms unnecessary. Tested in this manner were Rad24 and Rad50, as well as a selection of candidate proteins implicated in telomeric processing isolated from a large-scale genetic screen (see next section). This approach permitted isolation of a number of activities that fulfill the proposed criterion, indicating that multiple proteins can contribute to the processing observed at natural termini.

Several proteins have been implicated in the processing of chromosome termini. The first was Rad24, a component of the DNA-damage checkpoint pathway and part of an RFC-containing complex predicted to structurally resemble the clamp-loading machinery that acts during conventional DNA replication (Shimomura et al., 1998; Green et al., 2000; Naiki et al., 2000). Lydall and Weinert demonstrated that, in the absence of Rad24 function, the kinetics of

resection in strains with compromised end protection (*cdc13-1* strains at high temperature) were significantly altered. More specifically, resection of the C-rich strand of the chromosome was slowed dramatically when Rad24 function was abrogated. These data led the authors to suggest that Rad24 positively regulates a processing activity at the end (Lydall and Weinert, 1995; Booth et al., 2001). An interesting question remained: Rad24 itself is not known to possess the enzymatic activity required for processing, so what is the activity that it promotes? The authors proposed that Rad17 might provide this function, based on its homology to proteins with known exonucleolytic activity (*U. maydis* Rec1 and *S. pombe* Rad1) (Siede et al., 1996). However, the homology suggests that Rad17's nuclease activity proceeds in a 3'-5' direction, an orientation opposite to the polarity of the processing observed at the telomere (Booth et al., 2001). In addition, it was noted that C-strand resection was slowed but not abolished in *cdc13-1 rad24-Δ* strains at high temperatures, indicating that some other activity can promote degradation in the absence of Rad24.

To test whether Rad24 positively regulated a processing activity, we asked whether loss of Rad24 function would allow a *cdc13-Δ* strain to survive. Whereas a *cdc13-Δ* cell arrests at the large-budded stage, a *rad24-Δ cdc13-Δ* strains continues dividing, resulting in microcolony growth. To determine if this effect was specific to Rad24, other members of the epistasis group were tested in a similar fashion. Like Rad24, loss of Rad17 function resulted in microcolony growth. In addition, a partial rescue was observed with loss of Mec3 function, another member of the Rad24 epistasis group. However, the bypass provided by loss of Rad17 and Mec3 appeared to be less significant than that observed in *rad24-Δ cdc13-Δ* strains. According to the model presented in Chapter 2, Stn1 and Ten1 are components of the EPC, and the lethality of *stn1-Δ* and *ten1-Δ* mutants is due to the same mechanism that causes arrest of *cdc13-Δ* cells. Therefore, if crippled telomeric processing permits viability of *rad24-Δ cdc13-Δ* cells, a similar bypass of the essential function of the End Protection Complex should occur as well. As predicted, abrogation of *rad24-Δ* leads to microcolony growth in both *ten1-Δ* and *stn1-Δ* cells.

Rad50 was also examined in the microdissection assay to determine whether the MRX complex is involved in end processing. In contrast to the results observed at an artificial telomere (Diede and Gottschling, 2001), loss of Rad50 function could not bypass the arrest of *cdc13-Δ* cells. In addition, although loss of Rad24 can bypass lethality of *cdc13-Δ*, *ten1-Δ* and *stn1-Δ*, it is not able to bypass lethality due to loss of another essential protein involved in telomere maintenance, Tel2. Although little is known about Tel2, it has also been proposed to bind single-stranded G-rich telomeric DNA (Kota and Runge, 1999).

The bypass of *cdc13-Δ* lethality by abrogation of Rad24 function is only partial, and the microcolony eventually stops growing. However, rare colonies escape this fate and continue dividing to form normal, healthy appearing colonies. Analysis of the telomeric profile of these escaping colonies shows an altered telomeric profile very similar to that of a Rad52-dependent telomerase-negative survivor. The similarity in the telomeric profile suggests that these *rad24-Δ cdc13-Δ* escapers have utilized a recombination-based mechanism to maintain their telomeres in the absence of end protection, and that these terminal recombination events can somehow impart viability to a cell lacking the normal end protection machinery.

Experiments described in the next section indicated that ExoI might be involved in telomeric processing in the absence of Rad24 function. Briefly, mutants of ExoI were isolated which appeared to lead to enhanced growth of *rad24-Δ cdc13-Δ* cells. Encouraged by this result, and by prior observations demonstrating that Exo1's nuclease activity is of the correct orientation for telomeric processing (Fiorentini et al., 1997), we asked whether loss of Exo1

function could bestow growth on a *cdc13-Δ* strain even in the presence of functional Rad24. A doubly heterozygous *cdc13-Δ/CDC13 exo1-Δ/EXO1* strain was dissected and the four spore products analyzed for genotype and growth characteristics. Analysis of tetrad-type tetrads indicated that abrogation of Exo1 function is capable of promoting a partial bypass of *cdc13-Δ* lethality. On a gross level, it appeared as though the microcolonies formed were slightly smaller than those observed in *rad24-Δ cdc13-Δ* strains.

I reasoned that if Rad24 and Exo1 both played a role in end-processing, and did so through two different activities, then eliminating both might lead to more robust growth of *cdc13-Δ* cells. To examine this, I performed dissection and microcolony analysis on diploids triply heterozygous for the three mutations. Preliminary analysis suggests that the growth of *cdc13-Δ rad24-Δ exo1-Δ* strains is not grossly more robust than the double mutants alone. If more detailed epistasis confirms this result, it may indicate that Exo1 is in the same pathway of telomeric processing activities as Rad24.

Based on results from the same screen that isolated Exo1 as a putative end processing activity, my attention was also directed to a protein involved in nonsense-mediated decay, Nam7. Also known as Upf1, the protein plays a critical role in degradation of mRNA's that contain premature nonsense codons. In addition, several lines of evidence have indicated that the Upf proteins might be playing a more general role in global gene expression (Dahlseid et al., 1998; Lew et al., 1998). In support of this, microarray analysis indicated that loss of function of *UPF1*, *UPF2*, or *UPF3* leads to reproducible changes in transcript level of more than 375 different genes (362 are increased and 13 are decreased for expression levels) (see Culbertson web database: <http://144.92.19.47/default.htm> and Lelivelt and Culbertson, 1999). Our initial results indicated that abrogation of Nam7 function (in this case, by transposon-mediated mutagenesis) in a *rad24-Δ cdc13-Δ* strain led to nearly WT growth. To test whether this phenotype could be reconstituted in the absence of the transposon-mutagenized allele, I replaced the open-reading frame of *NAM7* with a nutritional marker by one-step PCR-mediated gene disruption. I then asked whether loss of Nam7 function could promote WT growth in a *rad24-Δ cdc13-Δ* strain. To do this, we subjected a triply mutant haploid strain covered by a WT *CDC13* plasmid (pVL438 *CEN URA3 CDC13*) to growth on 5FOA to force eviction of the covering plasmid. Confirming the previous results, loss of Nam7 function was sufficient to confer WT growth to a *rad24-Δ cdc13-Δ* strain that would normally support only microcolony growth. These healthy colonies displayed a rearranged telomeric profile when analyzed by Southern Blot.

In the case of Exo1, loss of function could promote growth of a *cdc13-Δ* even in the presence of Rad24. To ask whether this was true for Nam7 as well, we asked whether a *nam7-Δ cdc13-Δ* strain could form microcolonies. A doubly heterozygous strain was dissected and analyzed for microcolony growth of the *nam7-Δ cdc13-Δ* spores. Interestingly, unlike with Exo1, knocking out Nam7 could not promote division of *cdc13-Δ* spores, indicating that Rad24 must be absent for Nam7 to have a growth effect. Two reasons could exist for such an observation. First, Nam7 could affect levels of a processing activity that can access the chromosome termini only in the absence of Rad24. Alternatively, loss of Nam7 action could lead to upregulation of an activity that promotes recombination and therefore survival in *rad24-Δ cdc13-Δ* cells.

Regardless of the mechanism by which Nam7 exerts its influence over telomeric processes, it might be expected that such influence might manifest as alterations in telomere length. In fact, it has been reported that defects in Upf1, Upf2, and Upf3 can all lead to short telomeres (Lew et al., 1998). We subjected our *nam7-Δ* strains to the same analysis. Strains

lacking Nam7 function have short, but stable, telomeres. Epistasis analysis will be critical for understanding through which telomeric pathway loss of Nam7 affects telomere length.

#### A genetic screen designed to identify factors involved in telomeric processing

The work described above has indicated that, as predicted for a telomeric processing activity, loss of Rad24 promotes viability of *cdc13-Δ* cells. However, this bypass is only partial, indicating that at least one other enzyme can act at the telomere to promote C-strand resection when Rad24 is absent. If this reasoning is correct, than a genetic screen designed to isolate genes that, when mutated, enhance the growth of *rad24-Δ cdc13-Δ* strains should lead to identification of factors involved in telomeric processing. Transposon-mediated mutagenesis was chosen for a number of reasons, the most paramount being the increased likelihood of creating null alleles using this method. The library used has previously been published (Burns et al., 1994; Seifert et al., 1986).

Two major classes of mutants are predicted to be recovered from this screen. The first is composed of genes that, when mutated, enhance the recombination-based survival pathway of *rad24-Δ cdc13-Δ* cells (see above). Analysis of the telomere profiles of these "escapers" strongly suggests that they are able to grow by maintaining their telomeres in a recombination-dependent manner. Mutants that boost this recombination pathway, directly or indirectly, are expected to enhance the growth of the *rad24-Δ cdc13-Δ* strains, and therefore should be isolated in this screen. The second class of mutants expected from the screen included those mutants that contain disruptions in genes required for telomeric processing in the absence of Rad24, and it is this class that we are most interested in. Candidate proteins isolated in this manner need to be further tested for whether they can promote growth of *cdc13-Δ* cells only in the absence of Rad24, which would suggest that they function at the telomere only when normal processing activities are abrogated.

Figure 1 shows a conceptual presentation of this rationale. To test the predictions of the model, I subjected a *rad24-Δ cdc13-Δ/pVL910 (CEN CDC13 URA3 ADE2)* strain to transposon-mediated mutagenesis using an *mTn-LEU2* transposon-inserted library. Over 35,000 transformants were screened, representing a ~3.5X coverage of the genome. A total of 761 potential candidates were chosen based on the ability to support growth, above background, after loss of the plasmid bearing WT *CDC13* on 5FOA, and 544 of the 761 mutants retested this phenotype and became secondary candidates. Due to the microcolony growth phenotype of *rad24-Δ cdc13-Δ* cells, there was a significant background. To ensure no interesting candidates were lost, those mutants considered as displaying even marginal growth above background were chosen for further characterization. Due to the large number of primary candidates, three phenotypes were used for secondary screens. First, many of the candidates were tested, post-shuffle, for the presence of a red phenotype when plated on rich media with minimal adenine. Only those colonies that had lost the ability to synthesize both uradine and adenine would fulfill this criterium, and this step served as a way to eliminate those colonies that merely mutated the *URA* gene on the covering plasmid. In addition to testing the post-shuffle color of these mutants, we subjected 225 of the 544 primary candidates to telomere blot analysis (post-shuffle). Lastly, 220 strains were subjected to serial dilution and plating onto 5FOA to retest, again, their ability to rescue the poor growth phenotype of *rad24-Δ cdc13-Δ* strains. Any candidate exhibiting a red colony phenotype *or* an altered telomeric profile *or* was able to support growth on 5FOA was considered a secondary candidate and was carried on in the screen. Of the primary candidates

tested, 132 fulfilled at least one of the three secondary screen criteria. A comprehensive list of the secondary candidates to date can be found in Table 1.

Due to the simplicity of the method involved, we decided to attempt to determine the site of transposon integration in all 132 secondary candidates. To do this, we performed vectorette PCR on all of the mutants, and we were successful in determining at least one site of integration in 72/132 mutants. To confirm, independently, that the insertion site was correct, we designed a directed PCR test based on the predicted insertion site. Of the sixteen mutants tested in this manner, fifteen produced a band, confirming the validity of vectorette PCR as a method of determining the insertion site with confidence. It was also important to determine if any of the mutants had multiple insertions, which would complicate analysis when independent disruptions were created. To do so, a set of seven mutants, including those analyzed by directed PCR, were subjected to Southern Blot analysis, which served the dual purpose of providing a secondary confirmation of the insertion site based on predicted fragment sizes. Six of the seven mutants had only one insertion site. The seventh mutant, 3C-476, appeared to have 3 insertions.

It was immediately apparent, following the first round of vectorette PCR, that mutants were falling into distinct categories, as defined by their known/predicted function and/or their interacting protein partners. Table 1 is a comprehensive list of all of the interesting hits generated thus far. A candidate hit was deemed interesting if it met any of the following criteria: (a) possesses a known function at the telomere or demonstrates a two-hybrid or biochemical interaction with a protein known to play a role at the telomere (b) has a known or predicted role in chromatin structure or DNA repair, or demonstrated interaction with such a protein (c) had a telomere length defect when mutated as determined by analysis of the strains ordered from Research Genetics (independent of the initial screen candidate telomere length) (d) was hit multiple times, and with independent candidates (e) had an intriguing published mutant phenotype, such as in the case of *Lac1*, which leads to an alteration in life-span when mutated. (f) was a hit in a member of a pathway in which other components had been hit (for example, *UBR1* became a more interesting hit once *UBR2* was also hit) (g) was of completely unknown function, making it impossible to rule it out as an interesting hit.

Based on the criteria above, a few interesting classes of mutants emerged. One such class (Class I) included the following candidates: 3C-560 (*UBR1*), 3C-70 (*UBR2*), 3C-80 (*YJL184W*), and 1a-68 and 3C-112 (*NAS6*). All of these proteins play a known or suspected role in ubiquitin ligation and/or protein degradation, and all of the proteins interact with or are members of the proteasome. Such analysis indicates a link between these candidates, and lends credibility to their isolation in the screen. In addition, it is quite compelling that a deletion of either *UBR1* or *YJL184W* (in a *CDC13 RAD24* background) leads to a telomere length defect.

Another interesting class emerging from the screen involves chromatin remodelers, and includes candidates 3C-678b (*NOP4*), 3C-140 and 3C-197 (*HDA1*), 3C-552 (*BRE2*), 3C-227a (*RSC1*) and potentially 3C-173 (*BRE4*). Loss of Bre2 function leads to reproducibly short telomeres, and *BRE2* had recently been identified in a separate screen in lab to identify genes involved in telomere metabolism (Morris, and V. Lundblad, unpublished data). Mutations in *HDA1* (Histone deacetylase 1) lead to enhanced repression of subtelomeric chromatin (Rundlett et al., 1996). It can be envisioned that alterations in chromatin conformation leads to enhanced recombination in *rad24-Δ cdc13-Δ cells*, essentially promoting growth of the "rare" survivors previously characterized. Alternatively, it is possible that altered chromatin structure at the telomere makes the terminus (a) more accessible to activities that are normally excluded (inhibitors of processing) or (b) less accessible to activities normally present at the telomere.

Lastly, major alterations in chromatin structure can modify global transcription and hence this class may be exerting an effect at the telomere in a very indirect manner.

Also isolated from the screen were mutants 3C-154 (*MLH3*), 3C-489 (*PMS1*), and 3C-473 (*EXO1*). While *Exo1* is considered independently in the section above, it is notable that all three of these proteins are critical for proper functioning of the mismatch repair pathway. Recently, it has been published that defects in mismatch repair promote telomeric recombination, presumably due to a suppression of homeologous recombination by the wild-type pathway components (Rizki and Lundblad, 2001). Based on these results, it is reasonable to hypothesize that the enhanced growth of these screen candidates is due to increased homeologous recombination and subsequent promotion of the escape pathway employed by some *rad24-Δ cdc13-Δ* cells. If this is true, then deletion of these genes should promote growth of *cdc13-Δ* strains only in the absence of *Rad24*. However, this did not turn out to be the case for *Exo1*, which, as shown in the next section, can promote a partial bypass of *cdc13-Δ* lethality even when *Rad24* is present. Similar analysis of *MLH3* and *PMS1* is currently underway.

The next interesting group included those candidates whose insertions were located within genes already implicated in telomere maintenance. This group is composed of 3C-226 (*SGS1*), 3C-40 (*PIF1*), and 3C-592 (*EBS1*). *EBS1* was immediately interesting to us because it is a homolog of *EST1* (sharing 27% identity and 48% similarity over the open-reading frame), and the homology is greatest within the region encompassing the RNA recognition motif (RRM) of *EBS1*. Although it shares such high homology with the component of telomerase, *Ebs1* has no known function at the telomere. Loss of *Ebs1* function results in only a slight telomere length defect when deleted in an otherwise WT background (Zhou et al., 2000a). This candidate has a rearranged telomeric profile indicative of *Rad52*-dependent recombination at the terminus. This leaves open the possibility that 3C-592 is rescuing due to a boost in the *rad24-Δ cdc13-Δ* recombination-based escape pathway. Indeed, deletion of *pbs1-Δ* is not sufficient to promote viability of *cdc13-Δ* cells in the presence of *Rad24*.

Candidate 3C-226 has a robust growth phenotype when plated on 5FOA, indicating that the causative mutation strongly promotes growth of *rad24-Δ cdc13-Δ* cells. Vectorette PCR analysis identified a transposon insertion 2.1kb into the 4.3kb ORF of *SGS1*. There is much data to support a role for *Sgs1* in telomere maintenance, and this section does not aim to summarize all of the relevant literature. Briefly, *Sgs1* is a member of the RecQ family of DNA helicases, and loss of function mutants result in a hyperrecombination phenotype (Watt et al., 1996; Yamagata et al., 1998). Loss of the helicase function of *Sgs1* enhances senescence in telomerase negative cells, and results in exclusively Type I survivors, indicating that *Sgs1* is required for Type II survivor formation (Cohen and Sinclair, 2001; Johnson et al., 2001). In its WT form, *SGS1* acts to suppress homeologous recombination, and this action is redundant with the mismatch repair proteins (Myung et al., 2001). For these reasons, it seems likely that loss of *SGS1* function enhances growth of *rad24-Δ cdc13-Δ* due to a promotion of the recombination-based pathway of telomere maintenance, and the dependence of 3C-226's rescue ability on *Rad52* should allow confirmation of this.

Candidate 3C-40 became a secondary candidate owing to its ability to grow when plated as a serial dilution on 5FOA. Cloning of the disruption site indicated that an *mTn-LEU2* cassette has inserted 1.3kb into the 2.5kb ORF of *PIF1*. *Pif1* is a 5'-3' helicase, whose loss of function results in telomere elongation (Zhou et al., 2000b). In addition, *Pif1* activity represses telomerase action at the site of a de novo double strand break, and inhibits chromosome healing through telomere addition (Mangahas et al., 2001). It is unclear how, mechanistically, *Pif1* performs this

regulatory mechanism, but localization of the protein to telomeric DNA does support a direct role for the protein at the terminus (Zhou et al, 2000). A model in which Pif1 acts at the telomere to alter terminal structure, and in this way modulates the access of telomerase to the chromosome end can be envisioned. If this model is correct, the observed enhanced growth of 3C-40 may be a result of this altered telomeric structure. Additionally, the helicase function of Pif1 could be contributing to telomeric processing directly. Loss of such an activity would result in a mutant capable of supporting growth in the absence of end protection, and would fulfill the original screen prediction. Additional follow-up experimentation is needed to address these possibilities.

A number of other interesting mutants were isolated that did not appear to segregate into categories. These mutants include those with disruption in ORFs of unknown function, as well as independent candidates of known function, like Nam7 (see above section). A list of all of these candidates can be found in Table 1.

### **Key research accomplishments**

- Abrogation of Rad24 function imparts viability to cells that have lost the ability to protect the chromosome end
- The bypass of *cdc13-Δ* by loss of Rad24 function is only partial, and results in microcolony growth. Rare cells are able to escape this fate and grow into colonies of WT size and morphology, and they appear to do so by a recombination-based method of telomere maintenance
- Abolishing Exo1 function led to microcolony growth of *cdc13-Δ RAD24* strains, suggesting that Exo1 actively plays a role in wild-type telomeric processing
- Loss of Nam7 function promotes WT growth in *rad24-Δ cdc13-Δ* cells
- Design and implementation of a large-scale genetic screen to identify proteins involved in processing of the chromosome termini has been completed. 544 primary candidates, and 132 secondary candidates have been isolated in the screen.
- Isolation of a mutant allele of *EXO1*, which fulfills the original screen criteria and predictions, confirms the validity of the screening method.
- Generation of a large set of mutants that will serve as an excellent reagent for probing the activities that access chromosome termini.

### **Reportable Outcomes**

- The directed experiments described in the first half of the body of this report have been written as a chapter of my thesis with the intention of being submitted as a manuscript in the near future.
- I have completed my doctoral dissertation. Two of the chapters of that work are dedicated to those data summarized here.
- Graduate Student Symposium Platform Talk: Baylor College of Medicine, August 2001.

### **Conclusions**

In the past year, I have made considerable strides towards completing task 5 of my revised statement of work. As mentioned, the events upstream of Cdc13 binding to the telomere that make its presence essential remained elusive. Specifically, current models make use of a

hypothesized exonuclease that acts to process the telomere and provide a binding site for Cdc13. It is the identity of this proposed activity(s) that remained the largest missing piece of the puzzle of telomeric end protection. Experiments presented here were designed to identify this activity, and although the results from that work are still in the early stages, some conclusions can already be drawn. The most interesting conclusion is that multiple activities can access the chromosome terminus when end protection mechanisms are compromised. One such activity is controlled by Rad24, although the actual effector of the processing is still unknown. It remains to be seen which other gene products can function in this capacity at the terminus, and the collection of mutants described above should serve as an excellent reagent for accomplishing this goal.

Using a transposon-mediated mutagenesis technique, we were able to isolate mutants that enhanced cell survival in the absence of telomeric end protection. The project described here is currently ongoing, and much of the discussion regarding individual mutants or classes of mutants is addressed in the body of this text. Based on the analysis completed thus far, some general points regarding the efficacy of the screening method used can already be made.

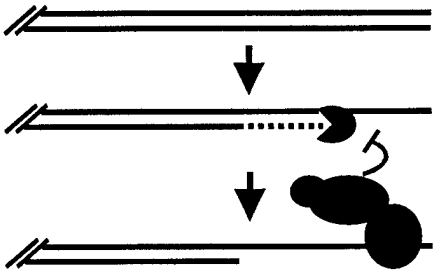
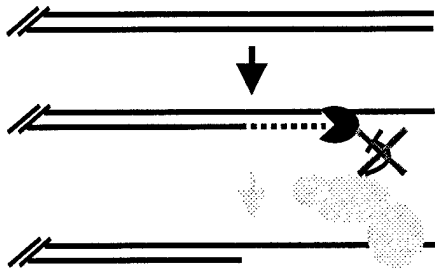
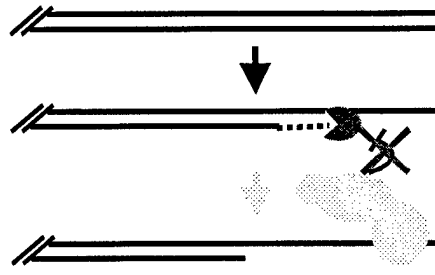
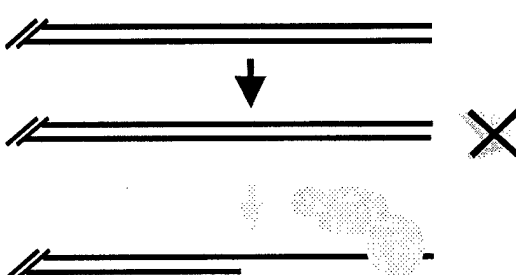
First, mutants that promote growth in the absence of both Cdc13 and Rad24 can, in fact, be isolated using this screening method. The ability of independently-created *Nam7* deletion alleles to rescue the poor growth phenotype of *rad24-Δ cdc13-Δ* strains proves the validity of the screening method, given that mutants with disruptions in *Nam7* were isolated in the initial screen. In this manner, the screen works well on a purely phenotypic level. Second, the screen can be used effectively to identify factors involved in cellular response to compromised end protection. This is proven by the *Exo1* test case. Alleles of *EXO1* were identified in the screen that led to a slightly enhanced growth of *rad24-Δ cdc13-Δ* cells. Upon directed testing, it was shown that deletion of *EXO1* could promote cell division in the absence of end protection (*cdc13-Δ exo1-Δ* strains form microcolonies). The ultimate test of whether the screen can isolate telomeric processing activities requires a further characterization of the exact enzymatic role of *Exo1* at the telomere. In other words, it will be important to test whether the kinetics of C-strand resection are altered in *cdc13-1 exo1-Δ* cells, as they are in *rad24-Δ cdc13-1* strains. In addition, studies designed to show that *Exo1* can be found at the telomere will aid in further characterization of the role of the protein at the telomere. In this manner, the penultimate test of whether we have truly identified a telomeric processing activity remains to be addressed.

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Figure 1

Genetic background	Pictorial model	Predicted or known outcome
<p><i>GENEX, RAD24</i></p> <p><i>CDC13</i> <i>STN1</i> <i>TEN1</i></p>		<p>viable</p>
<p><i>GENEX, RAD24</i></p> <p><i>cdc13-Δ</i> or <i>stn1-Δ</i> or <i>ten1-Δ</i></p>		<p>inviable</p>
<p><i>GENEX, rad24-Δ</i></p> <p><i>cdc13-Δ</i> or <i>stn1-Δ</i> or <i>ten1-Δ</i></p>		<p>microcolony growth</p>
<p><i>geneX-Δ rad24-Δ</i></p> <p><i>cdc13-Δ</i> or <i>stn1-Δ</i> or <i>ten1-Δ</i></p>		<p>viable</p>

**Figure 1:** Model for the screen designed to isolate telomeric processing activities. The first panel depicts the events that are hypothesized to occur in wild-type cells. The DNA molecule pictured is the daughter produced by leading strand synthesis, and therefore is shown as originally possessing a blunt terminus. A putative exonuclease, in this case controlled by Rad24 and shown by the pacman symbol, is then required to process the blunt end. This activity uncovers single-stranded G<sub>1-3</sub>T sequence that Cdc13 can bind, and it is this binding that permits recruitment of the End Protection Complex (EPC). A second activity, the product of *GENEX* transcription/translation, is shown as being functionally redundant with the Rad24-controlled processing activity. The EPC, consisting of at least Stn1, and Ten1, and recruited by Cdc13, then limits further action by the exonuclease. Further events downstream of these, including telomerase recruitment and C-strand fill-in, are not pictured. In the second panel, the consequence of compromised end protection is pictured. Inability to recruit the EPC, or failure of EPC function, leads to uninhibited processing by the exonuclease and eventual inviability of the cell. In the third panel, a model is presented for the microcolony growth observed in *rad24-Δ cdc13-Δ* cells. In these strains, the product of *GENEX* is present, and can partially compensate for the loss of Rad24, leading to slowed, but still functional processing. This partially crippled processing is eventually lethal. In the last panel, both exonuclease activities and EPC function are absent. In this case, no processing occurs, and therefore the essential function of the EPC becomes dispensable, and the cell remains viable. The potential consequence of a blunt-ended, unprocessed terminus is discussed in the text.

**Table 1:** Comprehensive list of all screen secondary candidates

<b>Candidate #</b>	<b>Color</b>	<b>Telomere Phenotype</b>	<b>Frogging Retest</b>	<b>Insertion site</b>
002A	white	slightly short	-/+	
002B	white	WT	++	<i>TEA1; AMD1</i>
003	red	rearranged	-	<i>YDR165W</i>
009A	red	rearranged.**	-	<i>YGR165W</i>
025	red	rearranged	-	<i>MCM21</i>
026	white	rearranged	++	
027A	white		++	
027B	white		+	<i>KAR5</i>
028	white		-/+	
030	white		+	<i>YGR111W; NAM7</i>
032	white		-/+	
037	white		-/+	
038	white		-/+	
040	white		++	<i>PIF1</i>
041B	white		-/+	
043	white	short	-	<i>LAC1</i>
048	white		-/+	
069	white		+	<i>AMD1</i>
070	white	short		<i>UBR2</i>
077	white	rearranged	-/+	
080	white	rearranged	-	<i>YJL184W</i>
082	red	rearranged	-/+	<i>ALG9</i>
084	red	rearranged	-/+	
102	pink		-	<i>YAR1</i>
105	white		-/+	
107	pink	inconclusive	-	<i>YPR071W</i>
108	white		-/+	
109	white		-/+	
111	red	short; rearranged	-	<i>DAP2</i>
112	white	slightly short	++	<i>NAS6; URA5</i>
114	white	inconclusive	-	
115	white		-/+	
118	white		-/+	
120	white		-/+	
124b			-/+	
129			-/+	
131			-/+	
132			-/+	
135	white		-/+	
137	RED		-	<i>YORO22C</i>
140	white	slightly short	-/+	<i>HDA1</i>
142b	white	inconclusive	++	

**Table 1:** continued

150			-/+	
154	RED		-	<i>MLH3</i>
157a	RED	short	+	<i>NAM7</i>
157b	RED	short	-	
171	white	slightly short	-	
173	PINK	WT	-	<i>BRE4</i>
181	RED	inconclusive	-	<i>YIP3; YNL043C</i>
187	PINK	slightly short	-	
197	RED		-	<i>HDA1</i>
202	RED		-	<i>NAM7, HSP104</i>
212	RED		-/+	<i>DFG16; YML081W; NAM7</i>
215	RED		-	<i>YDR003W</i>
217	white	inconclusive	-	<i>PAN3</i>
218	RED		-	
221	RED		-	<i>YPR117W</i>
225a	white	inconclusive		<i>IML1</i>
226	white	inconclusive	+	<i>SGS1</i>
227a	white	inconclusive	-	<i>RSC1</i>
227b	white	slightly short	-	
229	PINK		++	<i>NAM7</i>
235b	white	long		
243	white	rearranged		
249	white	rearranged-**	-	<i>SHE3</i>
250	white		++	<i>YGR122W, URA5</i>
267	white	rearranged-**	-	
273b			-/+	
278	white	slightly short	++	<i>YPR158W</i>
281	RED		-	<i>KIN4</i>
284	RED	slightly short	-	<i>HPT1</i>
285			-/+	
286			-/+	
290	white	rearranged-**	-	<i>CMP2</i>
294			-/+	
306	RED		-	
311	RED			
313	white	rearranged-**	-	
325		rearranged-**	-	<i>HYM1</i>
330	PINK	slightly short	-	
342a	white	short	-	<i>BAR1</i>
342b	white	short	-	
352	RED		-	<i>YPL108W; YPL109C; MLC2</i>
355	white	slightly short		
357	white	short; rearranged	-	<i>YGR130C</i>
361a	white	rearranged-**	-	<i>NCE102</i>

**Table 1:** continued

362	white	slightly short	-	<i>PAI3/ SIP18</i>
372		rearranged	-	
373b	white	rearranged	-	
385	RED		-	<i>YGR130C</i>
431	RED		-	<i>SIP1 )</i>
432	white	rearranged-**	-	<i>THI6</i>
433	white	short; rearranged	-	<i>CHRXII sequence</i>
443	white	Rearranged	-	
449	RED		-	
450	white	inconclusive		
463	white	rearranged	-	<i>YIL102c; YIL103W</i>
466	white	rearranged		<i>KIC1</i>
468	PINK	slightly short	-/+	<i>NPY1</i>
469	white	rearranged	-	
472	white	rearranged		<i>YPR196W</i>
473	PINK	rearranged	-	<i>EXO1</i>
475	white	rearranged	-/+	<i>YDR317W; MCM21</i>
477	RED		-	<i>YHR210C</i>
484	white	slightly short		<i>HYM1</i>
489	white	slightly short	-	<i>PMS1</i>
491a	RED	rearranged	+	<i>NAM7</i>
492		slightly short		
499	white	rearranged-**	-/+	
512	RED		-	<i>YPR196W</i>
525b	RED	slightly short	-	
527	white	slightly short		<i>YPR022C</i>
543	white	inconclusive		<i>TPS1</i>
552	PINK		-	<i>BRE2</i>
560	RED		-	<i>UBR1</i>
566	RED	short; rearranged		<i>NAM7;</i>
568a	white	Long	-	<i>SHE3</i>
580	RED		++	<i>NAM7</i>
588	RED	WT	-	
592	white	rearranged	-/+	<i>EBS1</i>
597	white	inconclusive		<i>BLM3</i>
600	white	inconclusive		
607	white	short		
625	white	inconclusive		<i>YBR270C; MUB1</i>
640	RED			<i>NAM7</i>
657	RED			<i>YBR270C; PST2</i>
662	PINK		-	
678b	PINK			<i>Nop4; RNY1</i>
688a	white	slightly short	-	<i>CSR2</i>
705	RED	rearranged	-/+	<i>PTC4</i>