Accurate chromosome segregation and transmission require the faithful execution of a number of processes. In the yeast Saccharomyces cerevisiae, the 16 chromosomes replicate once during S phase. A single microtubule is tethered at the centromere to the kinetochore, the DNA-protein complex that mediates the attachment of chromatids to the mitotic spindle. The chromatids segregate to opposite poles, resulting in retention of a full set of chromosomes in the mother cell and transmission of a full set of chromosomes to the daughter cell. The accurate execution and order of events that comprise the mitotic cell cycle is monitored and temporally controlled through distinct cellular processes at key points called checkpoints and is collectively referred to as checkpoint control.

We are interested in the functions of the kinetochore in chromosome segregation within the context of the cell cycle. The functional and structural relationships between centromeric DNA and the proteins that comprise the kinetochore have been extensively studied in S. cerevisiae. Centromeric DNA includes three conserved elements called CDEI (8 nucleotides (nt)), CDEII (78–86 nt), and CDEIII (25 nt), the latter two of which are essential for function (Fitzgerald-Hayes et al. 1982; Hieter et al. 1985; Gaudet and Fitzgerald-Hayes 1987; Hegemann et al. 1998). The CDEI element is bound by p39 (encoded by CBF1/CEP1/CPF1; Bram and Kornberg 1987; Baker et al. 1989; Cai and Davis 1990; Mellor et al. 1990). Although proteins that associate with CDEII have not been identified, the multiprotein CBF3 complex binds 56 nt of centromeric DNA, including CDEIII, to form the essential core of the kinetochore (Lechner and Carbon 1991). The in vitro assembly of the CBF3-CDEIII complex requires four CBF3 protein subunits that are called p110 (encoded by NDC10/CBF2/CTF14), p64 (encoded by CBF3 or CBF3B), p58 (encoded by CTF13), and p23 (encoded by SKP1) (Doheny et al. 1993; Goh and Kilmarin 1993; Jiang et al. 1993; Lechner 1994; Struninov et al. 1995; Bai et al. 1996; Connelly and Hieter 1996; Stemmman and Lechner 1996; Kaplan et al. 1997). Furthermore, in vitro crosslinking studies show that p58, p64, and p110 are in direct contact with CDEIII DNA, confirming earlier evidence that the direct CBF3-CDEIII contacts mini-
mally involve p64, which contains zinc finger motifs, and a second CBF3 subunit (Lechner 1994; Sorger et al. 1995; Espein et al. 1997).

The kinetochore has been implicated in checkpoint control prior to anaphase of the yeast mitotic cell cycle by the finding that mutations in centromeric DNA and mutations in three of the four essential genes encoding CBF3 subunits (CTF13, NDC10/CFB2/CTF14, and CEP3/CFB38) cause mitotic delay (Spencer and Hieter 1992; Do heny et al. 1993; Lechner 1994; Strunnikov et al. 1995; Bai et al. 1996; Conn elly and Hieter 1996). The mitotic delay requires the function of MAD and BUB genes previously identified because of their role in the mitotic spindle assembly checkpoint, thereby providing a link between the structural functions of the kinetochore and the regulatory functions of the cell cycle machinery (Hoyt et al. 1991; Li and Murray 1991; Wang and Burke 1995; Pangilinan and Spencer 1996). Moreover, CBF3-CDEII assembly is activated by p23-dependent phosphorylation of p58, which is also subject to ubiquitin-mediated degradation (Kaplan et al. 1997). These results are consistent with the suggestion that the concentration of p58 is limiting for in vitro complex assembly and in vivo kinetochore function (Doheny et al. 1993). Understanding how the expression, activation, and degradation of kinetochore subunits are regulated should provide insights into the mechanism of kinetochore assembly and its functional connection to checkpoint control.

The temperature-sensitive (Ts) mutation ctf13-30 impairs the fidelity of chromosome transmission and causes growth arrest at the G2/M boundary of the cell cycle at the restrictive temperature (Spencer et al. 1990; Doheny et al. 1993). In this article we show that a twofold increase in gene dosage of ctf13-30 leads to growth at the restrictive temperature, suggesting that increased expression of the Ts allele can restore kinetochore function and overcome cell cycle arrest. We identified four genes by analyzing extragenic suppressors of ctf13-30. Mutations in three of the four genes cause increased accumulation of ctf13-30 mRNA. These suppressors are alleles of genes previously identified as UPF1, UPF2, and UPF3, which are required for accelerated decay of mRNAs that contain a premature stop codon, so-called non-sense-mediated mRNA decay (Losson and Lacroute 1979; Leeds et al. 1991, 1992; Cui et al. 1995; He and Jacobson 1995; Lee and Culbertson 1995; Lee et al. 1995). Most importantly, the accumulation of wild-type CTF13 mRNA increases when UPF1 function is disrupted. We propose that the UPF genes are part of the natural repertoire of genes controlling CTF13 gene expression. Possible mechanisms for how the UPF-mediated decay affects CTF13 expression are discussed.

**MATERIALS AND METHODS**

**General methods:** The S. cerevisiae strains used in this study are described in Table 1. Plasmids are described in Table 2.

Media for growth and maintenance of yeast is described by Gaber and Culbertson (1982). Yeast transformations were performed using the lithium acetate method of Gietz et al. (1992) or by electroporation (Gray and Brendel 1992). Escherichia coli strains DH5a and DH12s were used for preparation of plasmid DNAs. Methods for growth, maintenance, and transformation of bacteria are described by Sambrook et al. (1989). Plasmid DNA was prepared from E. coli by the method of Birnboim and Doly (1979) as described by Sambrook et al. (1989) or by the method of Lee and Rashed (1990). Standard recombinant DNA techniques were used as described in Sambrook et al. (1989). All restriction enzymes were purchased from New England Biolabs (Beverly, MA) or Gibco BRL (Gaithersburg, MD). Chromosome mapping was accomplished by using a CHEF-DRII system (Bio-Rad, Hercules, CA) to separate chromosomes followed by hybridization to probes derived from cloned DNA.

**Cloning and analysis of ctf13-30:** The ctf13-30 allele was cloned by polymerase chain reaction (PCR). Yeast genomic DNA was prepared from strains YK33 and YK41, both of which carry the ctf13-30 mutation. DNA fragments were amplified by PCR using primers A (5'-GAT CCT CGA GGC AAC CAR TCG ACA ATG-3') and B (5'-ATA CGG GTT TTTC ACC-3'). Following incubation at 94°C for 2 min, cycling parameters were 94°C for 1 min, 50°C for 0.5 min, and 72°C for 2 min. The resulting products were gel purified, double digested with EcoRII-ClaII or BglII-ClaII, and cloned into plasmid pRS314 that was double-digested with SmaI-ClaII or BamHI-ClaII to create plasmids pUZ170 and pUZ199, respectively. DNA sequence analysis was performed by the chain termination method (Sanger et al. 1977) using double-stranded templates (Hattori and Sakaki 1986). Synthetic oligonucleotides complementary to CTF13 sequences (Doheny et al. 1993) were used as primers.

**Plasmid constructions:** Plasmids were constructed for use in gene replacement experiments. To construct plasmid pUZ157, which carries CTF13, a 1.5-kb BglII fragment carrying the TRP1 gene was inserted by blunt-end ligation into a unique BssHII site located ~0.4 kb downstream of the CTF13 open reading frame (ORF). To construct plasmid pUZ191, which carries ctf13-30, a 1.2-kb HindIII fragment carrying the TRP1 gene was blunt-end ligated into the same BssHII site downstream of CTF13. The EcoRII-ClaII fragment carrying CTF13 was replaced with the ctf13-30 EcoRII-ClaII fragment. To construct plasmid pUZ186, the EcoRII-ClaII fragment carrying CTF13 was replaced with the ctf13-30 EcoRII-ClaII fragment.

The CTF13 integrative plasmid YIpJD2 was generated by ligating a 3.0-kb BamHI-Sall DNA fragment from pUZ157 into the same sites in pRS305. The ctf13-30 integrative plasmid YIpJD3 was obtained by transforming E. coli with a ligation of Bsp120I-digested genomic DNA from strain JDY1. JDY1 contains YIpJD2 integrated at the CTF13 locus (see below), such that a Bsp120I digest liberates a fragment containing nearly all pRS305 sequences and the adjacent ctf13-30 allele. The BamHI-Bsp120I fragment in YIpJD3 includes 1.2 and 5.3 kb of sequence flanking the 5' and 3' sides of the ctf13-30 ORF, respectively. The presence of the ctf13-30 mutation was confirmed by DNA sequence analysis as described above.

Two multicopy CTF13 plasmids were constructed. A 3.6-kb SalI fragment containing CTF13 was ligated into YEp351 to generate YEpdD1. The insert in YEpD1 is oriented with the 3' end of CTF13 proximal to the EcoRI site in YEp351. A 3.4-kb SalI-EcoRI DNA fragment containing CTF13 and a 2.0-kb EcoRI-BglII fragment containing sequence immediately 3' of CTF13 were ligated into YEp351 double digested with Sall-BamHI to generate YEpdD2.

Three CTF13-CUP1 reporter plasmids were constructed. PfuI polymerase (Stratagene, La Jolla, CA) was used in a PCR.
with pRS315-CUP1(-) template DNA, and primers C (5'-AGA ATT CAT CGA AAT AGA TAT TAA G-3') and T7 (5'-TAA TAC GAC TCA CTA TAG GG-3'). pRS315-CUP1(-) was made by ligating a 1.3-kb genomic Sau3AI CUP1 DNA fragment derived from pYEcuP1(1.1) (a gift of Dennis J. Thiele, University of Michigan) into pRS315 digested with BamHI, such that the CUP1 3' end is proximal to T7 promoter sequences. The PCR product was digested with EcoRI and KpnI and ligated into the same sites in pRS426 to generate pRS426-CUP1. Integrity of the insert was confirmed by DNA sequence analysis. PfuI polymerase (Stratagene) was used in a PCR with YEpD1 template DNA, primer D (5'-AGA ATT CGT CGA CTA GGC C-3') and, either primer E (5'-AGA ATT CAA AAT GCA AAC CAC TC-3') or primer F (5'-TTT ATT TAA TTC GCT GAA AT CAA GCC ACA TGG AC-3'). The PCR products were digested with EcoRI or EcoRI and Pstl and ligated into the same respective sites of pRS426-CUP1 to generate YEpD7 and YEpD9, respectively. Plasmids were sequenced to confirm the integrity of the fusions. BamHI to Kpn (made blunt with T4 DNA polymerase) DNA fragments originating from these fusions were ligated into YEp351 digested with BamHI and SmaI to generate YEpD17 and YEpD18, respectively. To generate YEpD19, YEpD2 was digested first with CiaI, which was filled in using T4 DNA polymerase, and subsequently with BamHI. The resulting 2.6-kb DNA fragment containing CTFl3 was ligated into YEpD18 that was digested first with Pstl, which was made blunt with T4 DNA polymerase, and subsequently with BamHI.

**Strain construction: Starting with strain YK41-CF** (Table 1), the strains JDY1, JDY3, JDY5, and YPJ112 were constructed by integrative transformation and gene replacement. These strains are isogenic except for alterations at CTFl3 and UPF1. Strains JDY1 was constructed by transforming YK41-CF with MscI-digested YlpD2 plasmid DNA. Strain JDY3 was obtained as a Leu temperature-independent gene convertant of JDY1. Strains JDY5 and YPJ112 were constructed by single-step gene disruption (Rothstein 1992) and confers complete loss of UPF1 function. Strain JDY5 was similarly constructed by transformation of JDY2 with plasmid pUPF1-D that contains the upf1-44 allele, which is identical to upf1-62 except that it contains H153 in place of URA3. Strain YPJ113 was constructed by single-step gene disruption of UPF2 in YPJ108, a lys revertant of YK33, using a PCR product generated using PfuI polymerase (Stratagene), pRS303 plasmid DNA, primer G (5'-CTT ACT GTC GTG AGC TAG CGC TTT CAG TAC TCC TTA AAT GTG ATT GTA CGA GTG CAC-3') and primer H (5'-CTC TGA GAG TTT AGC AAC ACT CCG CTC ATT AAT CTC GTG GTG CGG TAT TCC ACA CGG-3'). The resulting allele contains H153 in place of the entire second exon of UPF2 and is termed upf1-34. All integrations and gene replacements were confirmed by Southern blotting (Southern 1975).

Strain JDY6 was constructed by transformation of YK41-CF with SalI-digested YlpD3 plasmid DNA. To confirm that JDY6 contains two copies of the ctf13-30 allele, a 3.6-kb SalI-SalI restriction fragment containing CTFl3 DNA (Figure 1) was used to probe JDY6 genomic DNA by Southern blotting. A 4.4-kb BamHI-PstI fragment located within the duplicated region was detected at exactly twice the abundance in strain JDY6 compared with YK41-CF. The abundance was determined by normalizing the amount of probe hybridized to the BamHI-PstI fragment relative to that hybridized to a 2.4-kb PstI-BamHI fragment located 5' of the duplicated region.

**Genetic analysis of ctf13-30 suppressors:** The parental strains used to isolate ctf13-30 suppressors each carry a nonessential chromosome fragment to assay for mitotic chromosome stabil-

ity. The presence of the chromosome fragment, which carries URA3, was maintained by growth in the absence of uracil. When plated for single colonies in the presence of uracil, loss of the chromosome fragment causes red sectors to form. The extent of sectoring serves as an indicator of the rate of chromosome loss (Hiet et al. 1985; see Figure 5 legend). To isolate suppressors, strains YK41, YK35, and YJP108 (Table 1) were grown in liquid cultures overnight at 25°. Aliquots (0.1 ml) containing 10⁶ cells were spread on standard YPD media plates and incubated at 37°. After 3-5 days incubation, temperature-resistant colonies appeared at a frequency of 1 per 10⁶ cells plated. Independent isolates were purified by replicating at 37° and analyzed using the colony color sectoring assay described above, by dominance tests, standard crosses, and complementation tests (Sherman et al. 1979; Hiet et al. 1985). The assignation of each suppressor to its respective locus was based on the combined results of segregation and complementation tests. For complementation tests, the suppressors were analyzed after transformation using plasmids carrying URA3 and UPFI, UPF2, or UPF3 (pRS316UPF1, pUZ178, and pLS17; Table 2). To accomplish this, Ura- derivatives of the suppressor strains were isolated after growth on YPD media by screening for Ura- colonies. The Ura- phenotype indicated loss of the chromosome fragment that carries URA3. Ura- suppressor strains were transformed with URA3 plasmids carrying each of the UPF genes and tested for the ability of the plasmids to complement the suppressor phenotype, which was indicated by lack of growth at 37°. The growth of strains carrying the suppressors was assayed using serial-dilution drop tests as described by Atkin et al. (1995).

**RNA methods:** Total RNA was prepared using the hot phenol method as described by Leeds et al. (1991). Pol y(A)- RNA was prepared from total RNA using a QUIAGEN (Chatsworth, CA) kit (Oligo(dT) mRNA mini kit). RNA samples were denatured in the presence of formaldehyde/formamide and fractionated on 1% agarose/16.2% formaldehyde gels or they were denatured by glyoxal/dimethyl sulfoxide (DMSO) treatment and fractionated on 1% agarose gels (Ausubel et al. 1993). Fractionated RNAs were transferred to GeneScreen Plus (Dupont, NEN Research Products, Boston) and analyzed by Northern blotting using riboprobes or DNA probes. The riboprobes were labeled during transcription by the incorporation of [α-32P]UTP (6000 Ci/mmol; DuPont, NEN Research Products) using the Gemini system (Promega Corp., Madison, WI) as directed. Specific activity was determined as suggested by the manufacturer, except that the percentage (α-32P)NTP incorporation was measured by counting samples taken before and after three sequential isopropanol precipitations. Riboprobe 1 is 321 nt in length and contains sequences complementary to nt 719-1011 within the CTFl3 ORF (Doherty et al. 1993). Riboprobe 2 is 267 nt in length and complementary to CUP1 sequences nt 66-1021 within the CTFl3 ORF. Total RNA was isolated from strains YK41, YK35, YK41-CTFI3, and YK41-CUP1(-) ( patent strain) by using a riboprobes, and this RNA was analyzed using the colony color sectoring assay described above.
fragments were denatured in 80% formamide loading buffer band values as described in results.

m 8 carrying rpb1-1 8 1022 J. N. Dahlseid et al.

CTF13 performed as follows. Oligonucleotide (1 pmol) was added resulting ratios for m pH 7.7, 4 m TTG GTT TAT CAG ACA G-3 with cold CTP during synthesis. A cocktail containing RNase for experimental variation. For blotting experiments, the val-

to molar activities [(counts per minute) per micromole]. The analyzed by Northern blotting. above. The reported half-life values are averages of half-lives to 8±10

ted from RNase protection experiments (see results). The resulting ratios for CTF13 and dtf13-30 mRNA from separate experiments were compared to assess the relative difference in abundance. The ratio for CTF13 mRNA was multiplied by the estimated abundance of U6 RNA (Li and Brow 1993) to determine the average copy number of mRNAs per cell in asynchronous log-phase cultures in synthetic media.

To determine the level of steady-state RNA accumulation, relative accumulation ratios were generated by dividing the average normalized value for an RNA from a mutant strain by the corresponding value for the RNA from an isogenic wild-type strain. CTF13 mRNA half-life data from upf1 null and wild-type strains were quantified and normalized as described above. The reported half-life values are averages of half-lives determined independently from three experiments, defined as the time at which 50% of the RNA remains. Standard deviation is reported as a measure of the variation observed. Standard deviations for accumulation ratios were propagated from standard deviations of average normalized values by standard statistical methods.

Figure 1.—Map of the CTF13 region. The CTF13 ORF is shown by the open arrow pointing in the direction of transcription. The locations of restriction sites mentioned in the text are shown on the same line. The distance indicated by the break in the line at the right end is 2.8 kb. The wild-type nucleotide and amino acid sequence surrounding the dtf13-30 mutation and the nature of the mutation are shown above the restriction map. Each line below the restriction map shows the length and location of DNA inserts in the plasmids mentioned in the text. Lollipops indicate that the DNA segment contains the dtf13-30 mutation. The location of DNA inserts containing wild-type TRP1 or URA3 genes is indicated by a triangle. The CUP1 ORF and noncoding regions are shown as a flat-pointed, open arrow and thin lines, respectively, and were fused to the CTF13 DNA indicated by the thicker lines and ORF (materials and methods).
RESULTS

The ctf13-30 mutation causes an amino acid substitution at position 146: To identify the ctf13-30 mutation, DNA fragments were independently amplified by PCR using DNA prepared from two mutant strains carrying ctf13-30 (materials and methods). The sequences of both fragments were compared with the sequence of CTF13 DNA. We identified a single mutation in the ORF, a G → A transition, which causes an amino acid substitution of lysine (AAG) for glutamic acid (GAG) at amino acid position 146 (Figure 1). Since the same mutation was identified in separate PCR amplifications, the observed change was not due to a PCR-induced error. One-step gene replacement (Rothstein 1983) was used to assess whether the Glu → Lys substitution at position 146 confers Ts growth similar to the authentic ctf13-30 allele. A BglII-Nspl fragment derived from plasmid pUZ157, containing both CTF13 and TRP1 from BglII to PstI, was isolated and used to transform a ctf13-30 strain to a Trp+ phenotype (see Figure 1). Of 14 Trp+ transformants resulting from gene replacement, 8 acquired the ability to grow at 37°, indicating that the BglII-PstI fragment supplies CTF13 function. Another experiment was performed in which a BglII-NsiI fragment derived from plasmid pUZ191, containing both ctf13-30 and URA3 from BglII to PstI, was isolated and used to transform a CTF13 strain to a Ura+ phenotype. Thirteen out of 185 Ura+ transformants resulting from gene replacement failed to grow at 37°. When a wild-type CTF13 gene was reintroduced on an autonomous plasmid into several of the 13 transformants, they regained the ability to grow at 37°. Together, these results indicate that the G → A mutation identified in PCR-amplified DNA confers Ts growth that is indistinguishable from the ctf13-30 mutation.

Growth at the restrictive temperature depends on the level of ctf13-30 mRNA accumulation: The results of a plasmid-shuffle experiment prompted us to test whether increased expression of the ctf13-30 allele might permit growth at 37°. The centromeric plasmid containing ctf13-30 and LEU2 (pUZ186) was introduced by transformation into strain YK113, which contains a chromosomal deletion of CTF13 and a multicopy 2μ plasmid carrying CTF13 and URA3. When Leu+ transformants were plated on media containing 5-fluorotic acid to select for loss of the plasmid carrying URA3, all resistant colonies purified through two rounds of selection grew at 37°. One plausible explanation for this result is that overexpression of the mutant allele might alleviate the growth defect. Overexpression could be achieved through unintended selection for extra centromeric plasmids or through elevated expression of the mutant allele from a single plasmid.

To test the effects of overexpression of the mutant allele on growth, strain JDY6 was constructed with two chromosomal copies of ctf13-30 located adjacent to each other at the ctf13 locus on chromosome 13. Southern blotting was used to confirm the presence of two ctf13-30 gene copies (materials and methods). The abundance of the ctf13-30 transcript was measured in RNA from strains with one (see YK41-CF+, Table 1) or two copies of ctf13-30 (JDY6) using RNase protection. The fragment remaining after RNase digestion was 1.5 ± 0.1 (n = 3) more abundant when protected by RNA from JDY6 compared to YK41-CF+ (Figure 2A). Although significantly elevated, the observed increase was lower than the twofold increase expected on the basis of Southern blotting. We do not yet understand why mRNA accumulation and gene dosage fail to correlate, but such discrepancies often signal the existence of a regulatory mechanism that limits expression.

The increase in expression of ctf13-30 in strain JDY6 causes growth at 35° and 37°, whereas strain YK41-CF+ failed to grow at both temperatures (Figure 2B). This result leads to the prediction that extragenic suppressors of ctf13-30 might include two classes, those that cause increased mRNA abundance and those that improve kinetochore function without changing mRNA abundance.

Suppressors of ctf13-30 map to four genetic loci: In order to identify extragenic suppressors of ctf13-30, 82 revertants that grew at 37° were isolated in ctf13-30 mutant strains YK41, YK35, and YJP108 (materials and methods; Table 1). Fifty of the revertants were plated at 30° to monitor the frequency of loss of a chromosome fragment present in these strains using a colony-selecting assay described previously (Hiet et al. 1985; see Figure 5 legend). All of the revertants tested exhibited a markedly reduced rate of loss of the chromosome fragment relative to its rate of loss in ctf13-30 parental strains, indicating that stable propagation of the chromosome fragment was restored in the revertants.

The revertants were mated to a strain carrying ctf13-30 and the resulting diploids were assayed for dominance by testing growth at 37°. Five of the 82 diploids grew robustly, suggesting the presence of dominant mutations. Four of the 5 dominant revertants were crossed to a CTF13 strain. All of the resulting tetrads segregated 4:0:4:0 for growth at 37°, implying close linkage to ctf13, a property expected of intragenic suppressors. Diploids derived from the 77 remaining revertants exhibited either no growth or poor growth with varying degrees of papillation, indicating a recessive phenotype. Twenty-five of the recessive suppressors were crossed to strains YJP101 or YJP102 in which the CTF13 gene was marked by the insertion of URA3 into the BsHI site immediately downstream of the CTF13 ORF (see Figure 1). When tetrads from each cross were analyzed, growth at 37° segregated independently of Ura+. This indicated that all of the 25 recessive mutations examined are extragenic suppressors of ctf13-30.

To estimate the number of genes represented in a
Yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>YK33</td>
<td>MAT α ctf13-30 ura3-52 lys2-801 ade2-101 trp1Δ1 his3Δ200 leu2Δ1 [CFIII (CEN3.L) SUP11 URA3]</td>
</tr>
<tr>
<td>YK35</td>
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</tr>
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<td>YK113</td>
<td>MAT α ura3-52 lys2-801 ade2-101 his3Δ200 trp1Δ1 ctf13-30::HIS3 [CFIII (RAD2.d) SUP11 TRP1] [2μCTF13 URA3]</td>
</tr>
<tr>
<td>YJP101</td>
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</tr>
<tr>
<td>YJP102</td>
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<td>MAT α ura3-52 leu2Δ1Δ112 trp1Δ289 his3Δ11,15 upf1Δ2::HIS3</td>
</tr>
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</table>

Extrachromosomal plasmids and chromosome fragments are enclosed in brackets. Chromosomally integrated DNA is indicated by a double colon. The plasmids listed in strains YK33, YK35, YK41, YK113, and YJP108 are described in Spencer et al. (1990). All other plasmids are described in Table 2.

YK41-Δ used throughout these studies is an isolate of YK41 lacking the nonessential chromosome fragment, but is otherwise identical to YK41.

A subset of suppressors, 3 suppressor strains derived from YJP108 were mated to 11 suppressor strains derived from YK41. Using this approach, 12 of the 14 suppressor strains could be assigned to one of two groups. The wild-type genes corresponding to each group were cloned by transforming strains carrying representative suppressors with a yeast genomic LEU2/CEN/ARS DNA library (F. Spencer and P. Hieter, unpublished data). Leu+ transformants were plated for single colonies on synthetic medium with uracil but lacking leucine and screened for increased chromosome loss using the colony-sectoring assay. Library plasmids that caused an increased frequency of red sectors were identified for both groups of suppressors, indicating complementation of the suppressor phenotype (white colonies).

Genes complementing the suppressor phenotype were identified using contour-clamped homogeneous electric field gels and grids of yeast genomic DNA fragments (Rivera et al. 1993). Inserts from the two library plasmids were shown to hybridize to DNA segments from chromosomes 13 and 8 that coincided with the known locations of two genes named UPF1 and UPF2 (NM22), respectively (Leeds et al. 1992; Cui et al. 1995; He and Jacobson 1995). The inserts in each plasmid recombined at the UPF1 and UPF2 loci by homologous recombination, as shown by analysis of tetrads from genetic crosses. To test whether the suppressor mutations affect UPF1 and UPF2 function, frameshift mutations were constructed by filling in an ApaI site in the UPF1 ORF and a BspEI site in the UPF2 ORF of the respective plasmids. The plasmids carrying the frameshift mutations failed to complement the corresponding recessive suppressors and respective recessive alleles of UPF1 and UPF2 described previously (Leeds et al. 1992). Together, these results indicate the suppressors are alleles of UPF1 and UPF2.

UPF1, UPF2, and UPF3 promote accelerated decay of pre-mRNAs and mRNAs that contain a premature stop codon (Leeds et al. 1991, 1992; Cui et al. 1995; He and Jacobson 1995; Lee and Leithard 1995; Lee et al. 1995). The finding that a subset of ctf13-30 suppressors are allelic to UPF1 and UPF2 prompted a complete analysis of all 77 recessive suppressors to see if other UPF genes were represented. Three plasmids called pRS316UPF1, pUZ178, and pLS17 were constructed (Table 2), each carrying the URA3 gene and either UPF1, UPF2, or UPF3, respectively. Ura+ derivatives of each of the suppressor strains (material and methods) were transformed with each of the three plasmids. The transformants were assayed for growth at 37°C. Complementation was indicated by failure to grow at this temperature. Thirty-eight strains failed to grow when transformed with pRS316UPF1, suggesting that the suppressors are in UPF1 (Table 3). Using similar reasoning, 24 of the suppressors are likely to be in UPF2, and 7 are in UPF3. Eight strains grew at 37°C regardless of the presence of any of the three plasmids. Pairwise crosses revealed that all eight suppressor mutations failed to
recombine and are therefore likely to be located in a single gene, which we have named ICK1 (interaction with Ctf13p or Kinetochore). Additional crosses with one representative ikc1 suppressor showed that the ikc1 locus is not genetically linked to the ctf13-30 locus or to any of the three known UPF loci. It remains unknown whether ICK1 corresponds to a previously identified gene.

**UPF suppressors affect CYH2 pre-mRNA and ctf13-30 mRNA accumulation:** Strains carrying suppressor mutations in UPF1, UPF2, UPF3, and ICK1 were examined to determine whether they interfere with nonsense-mediated mRNA decay. Accumulation of the CYH2 pre-mRNA was used as a diagnostic indicator of a functional nonsense-mediated mRNA decay pathway. CYH2, which codes for ribosomal protein L29 (Fried and Warner 1982), contains an intron that is inefficiently spliced from the transcript. Since the pre-mRNA contains a premature stop codon within the intron, its half-life and accumulation depend on whether the nonsense-mediated decay pathway is functional (He et al. 1993).

RNA from strains carrying representative suppressor mutations and the parental strain derivative YK41-CF was assayed by Northern blotting using a CYH2 probe (Figure 3A). The pre-mRNA was barely detectable in the parent strain YK41-CF, whereas a band corresponding to mature mRNA was readily detected. Comparison, prominent bands corresponding to both pre-mRNA and mature mRNA were detected in strains carrying suppressor mutations in UPF1, UPF2, or UPF3. In the experiment shown in Figure 3A (including duplicate lanes not shown), the pre-mRNA/mRNA ratios were 1.1 ± 0.1 (n = 2) for the upf1 suppressor, 0.7 ± 0.1 (n = 2) for the upf2 suppressor, and 1.0 ± 0.2 (n = 2) for the upf3 suppressor. A pre-mRNA/mRNA ratio of 1.3 ± 0.1 (n = 2) was observed using RNA from a strain carrying the null allele upf1Δ2 (not shown). These results indicate that all three upf suppressors cause an increase in abundance of CYH2 pre-mRNA from a barely detectable level to a level comparable with the mature mRNA. The nonsense-mediated mRNA decay pathway in these strains is therefore severely impaired. None of the eight ikc1 suppressors had an effect on the accumulation of CYH2 pre-mRNA (Figure 3A, data not shown), indicating that ICK1 plays no role in non-sense-mediated mRNA decay.

The accumulation of ctf13-30 mRNA was also assessed for UPF-dependent changes. RNA from the same suppressors examined in Figure 3A was assayed by Northern protection using riboprobe 1 (Figure 3B). Compared with strain YK41-CF, fold increases in the amount of protected riboprobe 1 were observed as follows: 1.9 ± 0.3 (n = 2) for the upf1 suppressor, 3.6 ± 0.4 (n = 2) for the upf2 suppressor, and 3.4 ± 0.4 (n = 2) for the upf3 suppressor. In all cases, the increase in mRNA abundance reflected by RNase protection is greater than the increase resulting from gene duplication, which is sufficient to confer growth at the restrictive temperature (see Figure 2). Using RNA from a strain carrying one of the ikc1 suppressors (Figure 3B), the relative amount of the protected fragment detected after RNase treatment was 1.1 ± 0.3 (n = 2) compared with the amount protected in YK41-CF. When strains carrying each of the seven other ikc1 suppressors were assayed in the same manner, the average amounts of protected riboprobe 1 ranged from 0.6 to 1.3. In contrast to suppressor mutations in UPF genes, these results suggest that sup-
pressor mutations in ICK1 have no effect on the accumulation of ctf13-30 RNA.

Loss of UPF function causes increased accumulation of ctf13-30 mRNA leading to improved growth and chromosome stability: In order to assess the effect of complete loss of UPF1 function on expression of ctf13-30, a strain (YJP112) isogenic to YK41-CF\(^{-}\) was constructed in which the complete UPF1 ORF was disrupted by gene replacement (materials and methods). Accumulation of ctf13-30 mRNA was assayed by RNase protection of \(^{32}\)P-labeled riboprobes 1 and 3 using RNA extracted from strain YK41-CF\(^{-}\) (ctf13-30 upf1) and YJP112 (ctf13-30 upf1\(-\Delta2\)) (Figure 4). The amount of riboprobe 1 remaining following RNase digestion was 2.1 \(\pm\) 0.4-fold more abundant (\(n = 8\)) when protected by RNA from strain YJP112 compared to YK41-CF\(^{-}\), confirming that the level of ctf13-30 mRNA accumulation depends on the presence of a functional UPF1 gene.

### TABLE 2
<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Vector</th>
<th>Yeast genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRS315(^a)</td>
<td>—</td>
<td>LEU2, CEN6, ARS4</td>
</tr>
<tr>
<td>pRS316(^b)</td>
<td>—</td>
<td>URA3, CEN6, ARS4</td>
</tr>
<tr>
<td>pu2170(^a)</td>
<td>pRS314(^a)</td>
<td>TRP1, CEN6, ARS4, ctf13-30(Eco47III-ClaI)</td>
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<tr>
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<td>pRS314(^b)</td>
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</tr>
<tr>
<td>pu2157(^a)</td>
<td>pRS314(^a)</td>
<td>TRP1, CEN6, ARS4, CTF13(XhoI-PstI):TRP1</td>
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<tr>
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<td>pRS314(^b)</td>
<td>TRP1, CEN6, ARS4, ctf13-30(XhoI-PstI):URA3</td>
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<tr>
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<td>pRS315</td>
<td>LEU2, CEN6, ARS4, ctf13-30(XhoI-PstI)</td>
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<tr>
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<td>pRS315</td>
<td>LEU2, CEN6, ARS4, UPF1(BamHI-EcoRI)</td>
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<tr>
<td>pRS316UPF1(^c)</td>
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<td>URA3, CEN6, ARS4, UPF1(BamHI-EcoRI)</td>
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<td>pRS305(^a)</td>
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<td>LEU2, 20(\mu) ori, CTF13(Sall-BggII)</td>
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<td>YEp351(^a)</td>
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<td>pBR322</td>
<td>upf1-Δ2::URA3</td>
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<tr>
<td>pUPF1-Δ4(^b)</td>
<td>pBR322</td>
<td>upf1-Δ4::HIS3</td>
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<tr>
<td>pKF11</td>
<td>pRS314</td>
<td>TRP1, CEN6, ARS4 CTF13(2.2-kb Sau3AI)</td>
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</table>

\(^a\) Sikorski and Hieter (1989).
\(^b\) This study.
\(^c\) Atkin et al. (1995).
\(^d\) Atkin et al. (1997).
\(^e\) The URA3 gene in YCP50 was replaced with LEU2 (J. Puziss and P. Hieter, unpublished data).
\(^f\) Hieter et al. (1986).
\(^g\) Position of junctions indicated by nucleotide (nt) location are in reference to the first nt of the ORF of the respective gene.
\(^h\) Christianson et al. (1992).
\(^i\) Leeds et al. (1992).
\(^j\) Dohney et al. (1993).

A double colon indicates an insertion of one gene into another. A single semicolon indicates an insertion of one gene adjacent to another.

### TABLE 3
Extragenic suppressors of ctf13-30

<table>
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<th>Gene</th>
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<th>YJP108</th>
<th>Total</th>
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<td>11</td>
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<tr>
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<td>7</td>
</tr>
<tr>
<td>ICK1</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>12</td>
<td>18</td>
<td>77</td>
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</tbody>
</table>

To assess the effect of complete loss of UPF1 function on suppression of ctf13-30, the growth of strain YJP112 (upf1\(-\Delta2\), one copy of ctf13-30) on complete synthetic medium was compared with strain YK41-CF\(^{-}\) (UPF1, one copy of ctf13-30), JDY6 (UPF1, two copies of ctf13-30), and JDY3 (UPF1 CTF13). YK41-CF\(^{-}\) failed to grow at 35\(^\circ\) and 37\(^\circ\). YJP112, JDY6, and JDY3 grew at both
Effect of UPF1 disruption on the accumulation of CTF13 and ctf13-30 mRNA. The PhosphorImage shows representative results of RNase protection (see Figure 2) using total RNA from isogenic strains JDY3 (CTF13 UPF1), JDYS (CTF13 upf1), YK41-CF− (ctf13-30 UPF1), and YJP112 (ctf13-30 upf1) as indicated with wild-type (+) or deleted (−) UPF1. Control lanes marked + and − contain control RNA with and without RNase treatment, respectively. The sizes of the protected riboprobes 1 and 3 were estimated by comparison with RNA molecular weight standards (left). The RNAs were resolved on a 5% polyacrylamide/8% urea gel.

Effect of suppressors on CYH2 pre-mRNA and ctf13-30 mRNA accumulation. (A) The autoradiogram shows representative results of a Northern blot of total RNA hybridized to DNA probes derived from PCR-generated template corresponding to nt 15–780 of the CYH2 ORF and intron (Kaufer et al. 1983). RNA was extracted from strains derived from YK41 carrying the indicated suppressors of ctf13-30 (upf3, upf2, upf1, and icxl) and from strain YK41-CF− (parent). The RNAs were resolved on a 1% agarose/16.2% formaldehyde gel. (B) The autoradiogram shows representative bands corresponding to riboprobe 1 and riboprobe 3 (materials and methods) using total RNA from the same strains as shown in A. Lanes marked + and − contain control RNA with and without RNase treatment, respectively. The sizes of the protected riboprobes 1 and 3 were estimated by comparison with RNA molecular weight standards (left). The RNAs were resolved on a 5% polyacrylamide/8% urea gel.

Loss of UPF1 function causes increased accumulation of wild-type CTF13 mRNA: To test whether mutations in UPF genes mediate a similar effect on the accumulation of CTF13 mRNA, RNase protection of 32P-labeled riboprobe 1 was used to determine the relative abundance of CTF13 mRNA in the absence of UPF1 function. The amount of the protected fragment remaining after RNase digestion was $3.6 \pm 0.7$-fold higher ($n = 8$) when the RNA used was from strain JDYS (CTF13 upf1-Δ2) relative to the isogenic strain JDY3 (CTF13 UPF1) (Figure 4). This suggests that the UPF-mediated effect on mRNA accumulation is independent of the ctf13-30 mutation.

U6 RNA, for which the complementary riboprobe serves as a control, is estimated to be present at 1000–2000 molecules per cell (Li and Brow 1993). Based on a comparison of the relative amounts of protected riboprobe 1 and riboprobe 3, U6 RNA is 2–4 × 10^4-fold more abundant than CTF13 mRNA in wild-type strains (see materials and methods). This suggests an abundance for CTF13 mRNA of significantly less than one molecule per cell. We also compared the accumulation data on ctf13-30 mRNA in strain YK41-CF− to that obtained above for CTF13 mRNA in the isogenic strain JDY3 (Figure 4). We infer from the RNase protection data that ctf13-30 mRNA is about fourfold more abundant than CTF13 mRNA (see materials and methods).
The CTF13 mRNA has a long extension at the 3' end:

To determine the size of the CTF13 mRNA hybridizing to riboprobe 1 in RNAase protection experiments, Northern blotting was used to detect CTF13 mRNA (Figure 6A). Poly(A)+ RNA was analyzed from strains JDY12 (UPF1) and JDY17 (upf1-Δ2). Using riboprobe 1, a single prominent band was detected in RNA from both strains with a calculated mobility corresponding to a 3.6-kb RNA. In the experiment shown, the detected band was 2.1 times more intense when the RNA was derived from strain JDY17 compared to JDY12, using ACT1 mRNA as a loading control. This indicated that the band detected by Northern blotting is CTF13 mRNA.

The RNA detected by Northern blotting was considerably larger than expected given the 1.4-kb intronless CTF13 ORF (Doherty et al. 1993). To resolve this discrepancy, we sought to overexpress the authentic CTF13 mRNA using multicopy 2μ plasmids. CTF13 multicopy 2μ plasmids YEpD1 and YEpD2 were constructed such that both contain ~1800 nt 5' and either 326 nt or 2222 nt 3' of the CTF13 ORF, respectively (Figure 1). We used Northern blotting to analyze total RNA from strain JDY17 transformed with either plasmid (Figure 6A). Using RNA from JDY17 carrying YEpD1 we detected several RNAs, none of which migrated with the same apparent mobility as the RNA detected in poly(A)+ RNA. This result might be explained by the lack of CTF13 transcriptional terminator sequences in the DNA flanking the ORF in YEpD1. To test this possibility, we used plasmid YEpD2, which contains more flanking DNA 3' of the CTF13 ORF. A single predominant RNA of the same apparent mobility as that present in poly(A)+ RNA was detected in RNA extracted from JDY17 carrying YEpD2 (Figure 6A). This indicates that the authentic CTF13 transcription terminator sequences are present in YEpD2 and lacking in YEpD1. Together these data suggest that the large size of the CTF13 mRNA is due to an extension of the mRNA on one side or both sides of the ORF.

To ascertain the relative lengths of 5' and 3' untranslated regions (utr), we mapped the CTF13 mRNA by oligonucleotide-directed RNAase H cleavage (Figure 6B). Total RNA from JDY17 carrying YEpD2 was hybridized to a series of oligonucleotides and treated with RNAase H (Materials and methods). Cleavages directed by oligonucleotides 1 and 2, both complementary to sequences in the 5' region of the CTF13 ORF, caused small relative shifts in the size of the detected RNAs. This indicates that the 5' end is in close proximity to the start codon. To estimate the location of the 5' end of the CTF13 mRNA, we used the size of the RNA detected after cleavage with oligonucleotide 3, estimated at 1520 nt, to infer a 5' utr of ~46 nt. This estimate places the region of transcription initiation 90 nt downstream of the 3' end of a perfect TATA sequence, which is also closest to the ORF. Cleavages directed by oligonucleotides 3 and 4, both complementary to sequences 3' of the CTF13 ORF, caused large and intermediate shifts in the size of the detected RNAs, respectively. Moreover, cleavage directed by oligonucleotide 5, which is complementary to sequence 2020 nt from the CTF13 stop codon, caused a slight but discernable shift in the size of the detected RNA. This indicates the CTF13 mRNA contains at least a 2-kb 3' end extension. The combined distance between the 3' end of oligonucleotide 5 and the 5' predicted end of the mRNA is 3523 nt. If an average length of poly(A) tail is present at the 3' end, the length of the mRNA is equivalent to the size of
The band we detect by Northern blotting. These results confirm that the CTF13 mRNA is 3.6 kb, including a 1.4-kb ORF and 5' and 3' utrs of ~46 nt and 2 kb, respectively.

**The half-life of wild-type CTF13 mRNA is not affected by loss of UPF1 function:** The half-life of CTF13 mRNA was determined using RNA extracted following termination of transcription in strains carrying rpb1-1, a Ts RNA polymerase II mutant (materials and methods). After temperature-shift from 25°C to 36°C, RNA was extracted from cultures of strains JDY7 (upf1-Δ2) transformed with either pRS315UPF1, which carries UPF1, or pRS315 and analyzed by RNase protection (Figure 7). At the time of temperature-shift (t = 0 min), the amount of riboprobe 1 detected after RNase digestion was 2.0 ± 0.6-fold higher (n = 3) in JDY7[pRS315] compared to JDY7[pRS315UPF1] (Figure 7A). When the half-life was derived from the logarithmic plot of percent RNA remaining vs. time after temperature-shift (Figure 7B), it was found to be 7.6 ± 1.4 min (n = 3) in JDY7[pRS315UPF1] and 8.4 ± 1.6 min (n = 3) in JDY7[pRS315]. These values are statistically indistinguishable by Student's t-test analysis, indicating that the increase in accumulation of CTF13 mRNA mediated by loss of UPF1 function cannot be accounted for by a change in the rate of mRNA decay.

The half-life analysis as assayed by RNase protection assumes that the decay of a fragment of CTF13 mRNA reflects that of the full-length mRNA. To confirm this, the half-life of the chromosomally expressed, full-length CTF13 mRNA was also determined by Northern blot analysis using rpb1-1 strains. After temperature-shift from 25°C to 36°C, RNA was extracted from cultures of strains JDY21 (upf1-Δ4) transformed with either pRS315UPF1, which carries UPF1, or pRS315, and analyzed by Northern blot (Figure 7). At the time of temperature-shift (t = 0 min), the amount of RNA detected by riboprobe 1 was 2.2 ± 0.4-fold higher (n = 3) in JDY21[pRS315UPF1] compared to JDY21[pRS315] (Figure 7C). When the half-life was derived from the logarithmic plot of percent RNA remaining vs. time after temperature-shift (Figure 7D), it was found to be 4.3 ± 0.4 min (n = 3) in JDY21[pRS315UPF1] and 4.3 ± 0.7 min (n = 3) in JDY21[pRS315]. These results confirm that CTF13 mRNA half-life remains unchanged when UPF1 is disrupted.

**CTF13-CUP1 promoter-reporter mRNAs fail to respond to loss of UPF1 function:** Since changes in CTF13 mRNA abundance cannot be explained by changes in mRNA half-life, we designed a reporter system to test effects on transcription. Three gene fusions were constructed to measure the effect of a upf1 mutation on the level of CUP1 reporter mRNAs transcribed via the promoter activity of various DNA fragments from the 5' side of CTF13 (Figure 1; materials and methods).

All three gene fusions include DNA from the BamHI site 1.2 kb 5' of the CTF13 translation start site up to the
Figure 7.—CTF13 mRNA half-life in UPF1 wild-type and upf1 null strains. The CTF13 mRNA half-life was determined by RNase protection (A and B) or by Northern blot (C and D) analysis of RNA extracted from samples collected at time intervals following termination of transcription as shown using riboprobe 1 (materials and methods). RNase protection of riboprobe 3 was used as a control. (A) The PhosphorImage shows the abundance of protected riboprobes using total RNA from strain JDY7 (upf1-Δ2) transformed with pRS315UPF1 (UPF1) and JDY7 transformed with pRS315 (upf1). Control lanes contain control RNA with (+) and without (−) RNase treatment, respectively. The sizes of the protected RNAs were estimated by comparison with RNA molecular weight standards (left). The positions of the protected riboprobes specific for the CTF13 mRNA and the U6 RNA and the molecular weight standards are indicated. (B) The values from three independent experiments are plotted as the log of the average percent RNA remaining vs. time. The error bars represent the standard deviation of the averages. Solid circles represent data using RNA from strain JDY7 transformed with pRS315UPF1. Open circles represent data from the same strain transformed with pRS315. (C) The autoradiogram shows high-specific activity riboprobe 1 (methods and materials) hybridized to total RNA from strain JDY21 (upf1-Δ4) transformed with pRS315UPF1 (UPF1) and JDY21 transformed with pRS315 (upf1). The position of the CTF13 mRNA is indicated. RNA samples were denatured by glyoxal/DMSO treatment prior to being resolved on a 1% agarose gel. (D) The values from three independent experiments are plotted as in B. Solid circles represent data using RNA from strain JDY21 transformed with pRS315UPF1. Open circles represent data from the same strain transformed with pRS315.

various points of fusion (Figure 1). YEpJD17 contains sequences encoding the entire CUP1 mRNA fused to CTF13 sequences 27 nt downstream of the putative TATA (110 nt upstream of translation start). YEpJD18 contains CTF13 sequences including the 5’ leader fused through the translation start codon to the entire CUP1 ORF and downstream sequences. YEpJD19 has CTF13 sequences including the 5’ leader and all of the coding
in UPF1 on reporter mRNA stability, the half-life was determined of reporter mRNAs produced from these fusions failed to show a maintained at a low level. Regulation of average, is consistent with the relative accumulation of the reporter mRNAs in the transcriptionally, suggesting that the level of Ctf13p may be regulated post-

region except the last 10 codons fused in frame to the seventh codon of the CUP1 mRNA. All fusions were carried on LEU2 multicopy 2μ plasmids to aid detection of reporter mRNAs driven by the weak UPF1(CUP1) promoter. Isogenic strains were made by transforming these strains with pRS316 or pRS136UPF1, which carries UPF1(Table 1). Total RNA from these strains was analyzed by Northern blotting (Figure 8). To control for variation in plasmid copy number and amount of RNA analyzed, the levels of the reporter mRNAs were normalized to the level of LEU2 mRNA transcribed from the reporter plasmid. Compared to the respective UPF1 wild-type strain, the relative accumulation of the reporter mRNAs in the upf1 mutant strain was 0.9 ± 0.1 (n = 3) for YEpJD17, 0.9 ± 0.1 (n = 3) for YEpJD18, and 1.0 ± 0.1 (n = 3) for YEpJD19 (Figure 8). In all three cases, reporter mRNAs produced from these fusions failed to show a steady-state increase in the upf1 mutant strain relative to wild type, suggesting that expression from the plasmid is independent of UPF1.

To confirm that there were no unanticipated effects on reporter mRNA stability, the half-life was determined in both UPF1 mutant and wild-type strains for one of the gene fusions. JDY22 (upf1-Δ4 cup1Δ) was transformed with YEpJD10, a URA3 multicopy 2μ plasmid with the gene fusion from YEpJD18. After temperature-shift from 25° to 36°, RNA was extracted from cultures of strains JDY22[YEpJD10] transformed with either pRS315UPF1, which carries UPF1, or pRS315 and analyzed by Northern blot analysis using the CUP1-specific riboprobe 3. When the half-life was derived from the logarithmic plot of percent RNA remaining vs. time after temperature-shift, it was found to be 6.9 ± 0.7 min (n = 3) in JDY22[YEpJD10, pRS315UPF1] and 6.1 ± 1.0 min (n = 3) in JDY22[YEpJD10, pRS315]. These values are statistically indistinguishable (Student’s t-test), indicating the absence of artifactual effects on the reporter mRNA stability.

**DISCUSSION**

CBF3 is a multisubunit complex that forms part of the kinetochore in *S. cerevisiae* (Lechner and Carbon 1991). Ctf13p (p58) is one of four subunits in this complex. We initially expected that extragenic suppressors of the temperature-sensitive ctf13-30 mutation might be located in genes that code either for other CBF3 subunits or for additional proteins that interact with the kinetochore. However, the results of a gene dosage experiment revealed that another type of suppressor was missing from the multicopy plasmids. When extragenic suppressors of ctf13-30 were analyzed, two types were found. Suppressors in the UPF1, UPF2, and UPF3 genes caused increased accumulation of ctf13-30 mRNA. Suppressors at the ik1 locus had no effect on the accumulation of ctf13-30 mRNA. It has not yet been determined if ICK1 encodes a CBF3 subunit or some other protein.

**Growth depends on the level of ctf13-30 mRNA accumulation:** Wild-type Ctf13p is limiting for in vitro CDEIII-CBF3 complex assembly and it was previously suggested that it may be limiting for in vivo kinetochore function (Doherty et al. 1995). Additionally, it has recently been shown that Ctf13p is the only CBF3 subunit that is subject to rapid ubiquitin-mediated degradation, suggesting that the level of Ctf13p may be regulated post-transcriptionally (Kaplan et al. 1997). Our finding that the CTF13 mRNA is present at very low abundance, probably much lower than one molecule per cell on average, is consistent with CTF13 gene expression being maintained at a low level. Regulation of CTF13 transcription may limit the assembly and consequently the function of the kinetochore complex. Furthermore, if the level of wild-type Ctf13p is regulated post-transcriptionally, any mutation resulting in decreased protein stability or function should compromise kinetochore function. Indeed, ctf13-30 mutant strains exhibit defects in kinetochore integrity in vivo, are Ts for growth, exhibit...
mitotic delay at the permissive temperature, and arrest at the G2/M phase of the cell cycle at the restrictive temperature (Doheny et al. 1993).

We present evidence that the level of ctf13-30 mRNA is fourfold higher than the wild-type mRNA at the permissive temperature. Because the level of wild-type Ctf13p most likely limits kinetochore function, we interpret our result to mean that the residual activity of the functionally impaired ctf13-30 protein is sufficient for function and growth at the permissive temperature only when overexpressed. Assembly of functional kinetochores, which is required for viability, appears to be kinetically favored by increased accumulation of the mutant mRNA at the permissive temperature. If the increase in ctf13-30 mRNA levels relative to wild type confers viability at the permissive temperature, then a further increase in the mutant mRNA level might similarly confer growth at the restrictive temperature. Our data are consistent with this idea. We observe that increased ctf13-30 mRNA accumulation resulting from gene duplication or from the presence of suppressor mutations in the UPF genes both result in growth at the restrictive temperature. All of our data are consistent with the hypothesis that growth of a strain carrying the ctf13-30 mutant allele is dependent on ctf13-30 mRNA abundance.

It is possible that the increase in accumulation of ctf13-30 mRNA at the permissive temperature relative to wild type is due to an increase in the intrinsic half-life of the mutant mRNA. Missense mutations typically do not alter the stability of mRNA. Two alternative explanations might account for the increased accumulation of the ctf13-30 mRNA. An increase in either the rate or duration of transcription would result in increased ctf13-30 mRNA levels. If the induction of ctf13-30 expression is temporally restricted during the cell cycle, lengthening the duration of this period would allow for greater mRNA accumulation. Although nothing is known about the rate of ctf13 transcription, the accumulation of ctf13-30 mRNA could be explained by the finding that strains carrying ctf13-30 exhibit mitotic delay (Doheny et al. 1993). Mitotic decay in ctf13-30 strains might allow a temporal lag of sufficient duration to cause an apparent increase in expression and therefore explain the accumulation of the ctf13-30 mRNA relative to wild type.

The correlation between increased mRNA accumulation and growth might be explained by the suggestion that assembly of functional kinetochores and/or their attachment to the spindle may be monitored by surveillance at a cell cycle checkpoint (Lee et al. 1991, 1992; Cui et al. 1995; He and Jacobson 1995; Lee and Culbertson 1995; Lee et al. 1995) and at present have not been shown to be involved in other cellular functions. Second, suppressor mutations of ctf13-30 were found in all three UPF genes, suggesting that suppression of the UPF-mediated mRNA decay pathway, and not a particular UPF gene, is responsible for suppression. Lastly, we found that ctf13-30 suppressor mutations in all three UPF genes affect the accumulation of CYH2 pre-mRNA, another RNA target of UPF-mediated decay. Since the effects of the UPF suppressors are not limited to ctf13-30 mRNA and involve CYH2 pre-mRNA as well, we interpret this to mean that suppressors probably cause a general impairment of nonsense-mediated decay in a manner that affects all target RNAs of the UPF-mediated decay pathway.

CTF13 mRNA may be one of several natural mRNAs affected by UPF-mediated mRNA decay. While the ma-
majority of wild-type RNAs in yeast do not appear to be affected by the UPF-mediated mRNA decay pathway (Leeds et al. 1991, 1992), three classes of natural RNAs that are affected have been identified. The first class includes the inefficiently spliced pre-mRNA products of the CYH2, RP51B, and MER2 genes. These pre-mRNAs are stabilized two- to fivefold when UPF1 is inactivated (He et al. 1993), indicating that they are direct targets of UPF-mediated decay. Pre-mRNA targets of UPF-mediated decay are analogous to nonsense mutant mRNAs, because they contain in-frame premature nonsense codons within their unspliced introns (He et al. 1993).

A second class of natural RNAs affected by UPF-mediated decay is exemplified by the URA3 mRNA. In the absence of UPF1 function, the abundance of URA3 mRNA increases without a corresponding change in half-life (Leeds et al. 1991), indicating it is indirectly affected by UPF-mediated decay. Reportedly, the mRNAs encoded by URA1 and URA4, but not URA5, also accumulate in a upf1 mutant strain (cited in Pel t z and Jacobson 1993). This result can be explained if the UPF-dependent accumulation of URA3 mRNA is mediated by the activity of PPR1, which codes for a transcriptional activator of URA1, URA3, and URA4, but not of URA5 (Lisson et al. 1983). Consistent with this idea, we have observed that the PPR1 mRNA accumulates to a higher level in upf mutant strains (M. Le l i v et al. and M. R. Cul bert s o n, unpublished data). In the absence of UPF1 function, an increase in the abundance of PPR1 mRNA presumably leads to increased abundance of the Ppr1p activator, resulting in increased transcription of URA1, URA3, and URA4. Therefore, some natural mRNAs may be indirectly affected when their expression is modulated by factors expressed from mRNAs that respond to inactivation of UPF-mediated decay. According to this view, a third class of mRNAs that code for regulatory proteins should exist. Members of this class would be expected to be direct substrates of UPF-mediated decay and should exhibit a change in half-life in response to inactivation of the UPF genes. At present, the best potential candidate for a member of this class is the PPR1 mRNA.

The CTF13 gene lacks an intron, so the mRNA cannot be targeted for UPF-mediated decay through a mechanism that involves inefficient splicing. We performed experiments to determine whether the CTF13 mRNA is a direct or an indirect natural mRNA target of UPF-mediated decay. In order to accomplish this, the CTF13 mRNA was first characterized. We detected a single 3.6-kb poly(A)+ CTF13 mRNA by Northern blotting. Using oligonucleotide-directed RNase H cleavage, we show that the mRNA we detect is the CTF13 mRNA and that its unexpectedly large apparent size reflects its actual size. The additional length of the mRNA is due to the presence of an ~2-kb-long extension at the 3’ end, which is atypical for yeast mRNAs. Nonetheless, we do not believe that this feature of the mRNA causes it to be subject to accelerated decay mediated by the UPF genes. Using both RNase protection and Northern blot analysis, we observed that loss of UPF1 function caused no change in the half-life of CTF13 mRNA expressed from the wild-type chromosomal gene at its normal location. On the basis of these data, we conclude that the CTF13 mRNA belongs to the class of natural mRNAs that are indirectly affected by UPF-mediated decay.

We generated three different promoter-reporter gene fusions to determine whether a UPF-dependent change in transcription was responsible for the CTF13 mRNA accumulation. In no case did we observe an increase in reporter mRNA accumulation when the UPF-mediated decay pathway was inactivated. Each fusion contains ample (1.2 kb) CTF13 5’ noncoding DNA and one fusion contains additional DNA encompassing all but 30 nt of the CTF13 ORF. It is unlikely that we have missed a conventional upstream or downstream element for a transcription factor encoded by an mRNA substrate of UPF-mediated decay.

We imagine two possible reasons for why the reporters might not have detected a change in the expression of CTF13 mRNA. One reason for the lack of response of the reporters is that CTF13 expression may be regulated by an abundant factor encoded by an mRNA that is a direct substrate of UPF-mediated decay. The multicopy plasmids we used in the reporter experiments might titrate downward the apparent activity of such a factor. We expect that titration should reduce but not eliminate the difference in magnitude of reporter mRNA accumulation. Since no change was observed, the effect of upf mutations on CTF13 expression may not parallel their effect on URA3.

Another possibility stems from the intriguing discovery that the UPF-mediated decay pathway is required for telomere functions, including the establishment and/or maintenance of telomeric chromatin as indicated by reduced telomeric silencing in upf mutant strains (Lew et al. 1998). It is tempting to speculate that an mRNA targeted by UPF-mediated decay might encode a protein generally involved in gene silencing or in general functions affecting chromatin structure (including telomeric). Implicit to this speculation is that CTF13 gene expression may be sensitive to effects on silencing or chromatin structure. In this scenario, an increase in stability of the target mRNA would interfere with silencing, or alter chromatin dynamics, and promote increased CTF13 mRNA expression. The results of our reporter mRNA analysis are consistent with this idea. If chromosomal context were important for the effect of silencing or chromatin dynamics on CTF13 gene expression, reporters expressed from plasmids might fail to reflect a UPF-dependent change in reporter mRNA accumulation. Nevertheless, we find it intriguing that disruption of UPF-mediated decay affects chromosomes at both the centromere and the telomere. It is possible the observed effects could involve more than one mRNA.
target, in which case the actual targets of UPF-mediated decay may be more than one step removed from CTF13 mRNA expression.

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