Accumulation of mRNA Coding for the Ctf13p Kinetochore Subunit of *Saccharomyces cerevisiae* Depends on the Same Factors That Promote Rapid Decay of Nonsense mRNAs

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ABSTRACT

The *CTF13* gene codes for a subunit of the kinetochore in *Saccharomyces cerevisiae*. The temperature-sensitive mutation *ctf13-30*, which confers reduced fidelity of chromosome transmission, is a G → A transition causing an amino acid substitution of Lys for Glu$_{146}$. Strains carrying one chromosomal copy of *ctf13-30* fail to grow at the restrictive temperature, whereas a haploid strain carrying two copies of *ctf13-30* can grow. Four genes, *UPF1*, *UPF2*, *UPF3*, and *ICK1*, were represented among extragenic suppressors of *ctf13-30*. The *UPF* genes encode proteins that promote rapid decay of pre-mRNAs and mRNAs containing a premature stop codon. Suppressor mutations in these genes restore kinetochore function by causing increased accumulation of *ctf13-30* mRNA. They also cause increased accumulation of *CYH2* pre-mRNA, which is a natural target of *UPF*-mediated decay. Mutations in *ICK1* restore kinetochore function but have no effect on *ctf13-30* mRNA or *CYH2* pre-mRNA accumulation. Most importantly, loss of *UPF1* function causes increased accumulation of wild-type *CTF13* mRNA but has no effect on the mRNA half-life. We propose that *UPF*-mediated decay modulates the mRNA level of one or more factors involved in *CTF13* mRNA expression.

$\mathbf{A}$CCURATE 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.* 1988). 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 *CEP3/CBF3B*), p58 (encoded by *CTF13*), and p23 (encoded by *SKP1*) (Doheny *et al.* 1993; Goh and Kilmartin 1993; Jiang *et al.* 1993; Lechner 1994; Strunnikov *et al.* 1995; Bai *et al.* 1996; Connelly and Hieter 1996; Stemmann 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; Espelin *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/CBF2/CTF14,* and *CEP3/CBF3B*) cause mitotic delay (Spencer and Hieter 1992; Doheny *et al.* 1993; Lechner 1994; Strunnikov *et al.* 1995; Bai *et al.* 1996; Connelly 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-*CDEIII* 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 G_2/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 (Grey and Brendel 1992). *Escherichia coli* strains DH5α 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 Rasheed (1990). Standard recombinant DNA techniques were used as described in Sambrook *et al.* (1989). All restriction enzymes were purchased from either 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 GCG AAG CAC TCG ACA ATG-3′) and B (5′-ATA CCG CCG GTT TTC CAC-3′). Following incubation at 94° for 2 min, the cycling parameters were 94° for 1 min, 50° for 0.5 min, and 72° for 2 min. The resulting products were gel purified, double digested with *Eco*47III-*Cla*I or *Bgl*II-*Cla*I, and cloned into plasmid pRS314 that was double-digested with *Sma*I-*Cla*I or *Bam*HI-*Cla*I 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 *Bgl*II fragment carrying the *TRP1* gene was inserted by blunt-end ligation into a unique *Bss*HII 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 *Hin*dIII fragment carrying the *URA3* gene was blunt-end ligated into the same *Bss*HII site downstream of *CTF13*. The *Eco*47III-*Cla*I fragment carrying *CTF13* was replaced with the *ctf13-30 Eco*47III-*Cla*I fragment. To construct plasmid pUZ186, the *Eco*47III-*Cla*I fragment carrying *CTF13* was replaced with the *ctf13-30 Eco*47III-*Cla*I fragment.

The *CTF13* integrative plasmid YIpJD2 was generated by ligating a 3.0-kb *Bam*HI-*Sal*I 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 *Bsp*120I-digested genomic DNA from strain JDY1. JYD1 contains YIpJD2 integrated at the *CTF13* locus (see below), such that a *Bsp*120I digest liberates a fragment containing nearly all pRS305 sequences and the adjacent *ctf13-30* allele. The *Bam*HI-*Bsp*120I 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 *Sal*I fragment containing *CTF13* was ligated into YEp351 to generate YEpJD1. The insert in YEpJD1 is oriented with the 3′ end of *CTF13* proximal to the *Eco*RI site in YEp351. A 3.4-kb *Sal*I-*Eco*RI DNA fragment containing *CTF13* and a 2.0-kb *Eco*RI-*Bgl*II fragment containing sequence immediately 3′ of *CTF13* were ligated into YEp351 double digested with *Sal*I-*Bam*HI to generate YEpJD2.

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 *Sau*3AI *CUP1* DNA fragment derived from pYeCUP(1.1) (a gift of Dennis J. Thiele, University of Michigan) into pRS315 digested with *Bam*HI, such that the *CUP1* 3′ end is proximal to T7 promotor sequences. The PCR product was digested with *Eco*RI and *Kpn*I 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 YEpJD1 template DNA, primer D (5′-AGA ATT CGT CGA CTG CTA GGC C-3′), and either primer E (5′-AGA ATT CAA AAT GCA AAG CAC TC-3′) or primer F (5′-TTT AAT TAA TTC GCT GAA CAT CAA GCG ACA TGG AC-3′). The PCR products were digested with *Eco*RI or *Eco*RI and *Pac*I and ligated into the same respective sites of pRS426-CUP1 to generate YEpJD7 and YEpJD9, respectively. Plasmids were sequenced to confirm the integrity of the fusions. *Bam*HI to *Kpn*I (made blunt with T4 DNA polymerase) DNA fragments originating from these fusions were ligated into YEp351 digested with *Bam*HI and *Sma*I to generate YEpJD17 and YEpJD18, respectively. To generate YEpJD19, YIpJD2 was digested first with *Cla*I, which was filled in using T4 DNA polymerase, and subsequently with *Bam*HI. The resulting 2.6-kb DNA fragment containing *CTF13* was ligated into YEpJD18 that was digested first with *Pac*I, which was made blunt with T4 DNA polymerase, and subsequently with *Bam*HI.

Strain construction: Starting with strain YK41-CF⁻ (Table 1), the strains JDY1, JDY3, JDY5, and YJP112 were constructed by integrative transformation and gene replacement. These strains are isogenic except for alterations at *CTF13* and *UPF1*. Strains JDY1 was constructed by transforming YK41-CF⁻ with *Msc*I-digested YIpJD2 plasmid DNA. Strain JDY3 was obtained as a Leu⁻ temperature-independent gene convertant of JDY1. Strains JDY5 and YJP112 were constructed by single-step gene disruption (Rothstein 1983) of *UPF1* in JDY3 and YK41-CF⁻, respectively, using plasmid pPL65 carrying *upf1-Δ2*. The *upf1-Δ2* allele used throughout these studies is described in Leeds *et al.* (1992) and confers complete loss of *UPF1* function. Strain JDY17 was similarly constructed by transformation of JDY12 with plasmid pUPF1-Δ4 that contains the *upf1-Δ4* allele, which is identical to *upf1-Δ2* except that it contains *HIS3* in place of *URA3*. Strain YJP113 was constructed by single-step gene disruption of *UPF2* in YJP108, a Lys⁺ revertant of YK33, using a PCR product generated using PfuI polymerase (Stratagene), pRS303 plasmid DNA, primer G (5′-CTT ACT GTG GCC AGA TCG GCC TTT CAG TAC TTC TAA GGT TAG ATT GTA CTG AGA GTG CAC-3′) and primer H (5′-TCG TGA GAG TTG ACT AAC ACT CCG CTC TTT AAT CTC CTG CTG GTG CGG TAT TTC ACA CCG-3′). The resulting allele contains *HIS3* in place of the entire second exon of *UPF2* and is termed *upf2-Δ1*. All integrations and gene replacements were confirmed by Southern blotting (Southern 1975).

Strain JDY6 was constructed by transformation of YK41-CF⁻ with *Sal*I-digested YIpJD3 plasmid DNA. To confirm that JDY6 contains two copies of the *ctf13-30* allele, a 3.6-kb *Sal*I-*Sal*I restriction fragment containing *CTF13* DNA (Figure 1) was used to probe JDY6 genomic DNA by Southern blotting. A 4.4-kb *Bam*HI-*Pst*I 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 *Bam*HI-*Pst*I fragment relative to that hybridized to a 2.4-kb *Pst*I-*Bam*HI 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 (Hieter *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 10⁷ 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 10⁷ cells plated. Independent isolates were purified by replating at 37° and analyzed using the colony color sectoring assay described above, by dominance tests, standard crosses, and complementation tests (Sherman *et al.* 1979; Hieter *et al.* 1985). The assignment 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 *UPF1, 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). Poly(A)⁺ RNA was prepared from total RNA using a QIAGEN (Chatsworth, CA) Oligotex(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]CTP (800 Ci/mmol; Amersham Life Science, Arlington Heights, IL) or, for the Northern blot analysis in Figure 7, [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 *CTF13* ORF (Doheny *et al.* 1993). Riboprobe 2 is 267 nt in length and complementary to *CUP1* sequences 66 nt upstream through 12 nt downstream of the ORF. Using template DNA prepared by restriction digest or PCR, *ACT1, LEU2,* and *CYH2* probes were prepared and used in hybridizations as described previously (Atkin *et al.* 1995).

Riboprobe 1 was used to determine the accumulation and half-life of *CTF13* mRNA by Northern blot analysis and the accumulation of *CTF13* and *ctf13-30* mRNA by RNase protection analysis. For RNase protection, 10 μg of total RNA was mixed with a fourfold excess of riboprobe 1 and a twofold excess of riboprobe 3 and hybridized at 53° for 18–20 hr using an Ambion (Austin, TX) RPA II RNase protection kit according to instructions. The 165-nt riboprobe 3 was used to detect a 112 nt fragment complementary to U6 RNA (Brow and Guthrie 1988) as a control. This RNA was chosen because it shows no significant decay during the time course of our experiments (Moenne *et al.* 1990). Given the relatively high
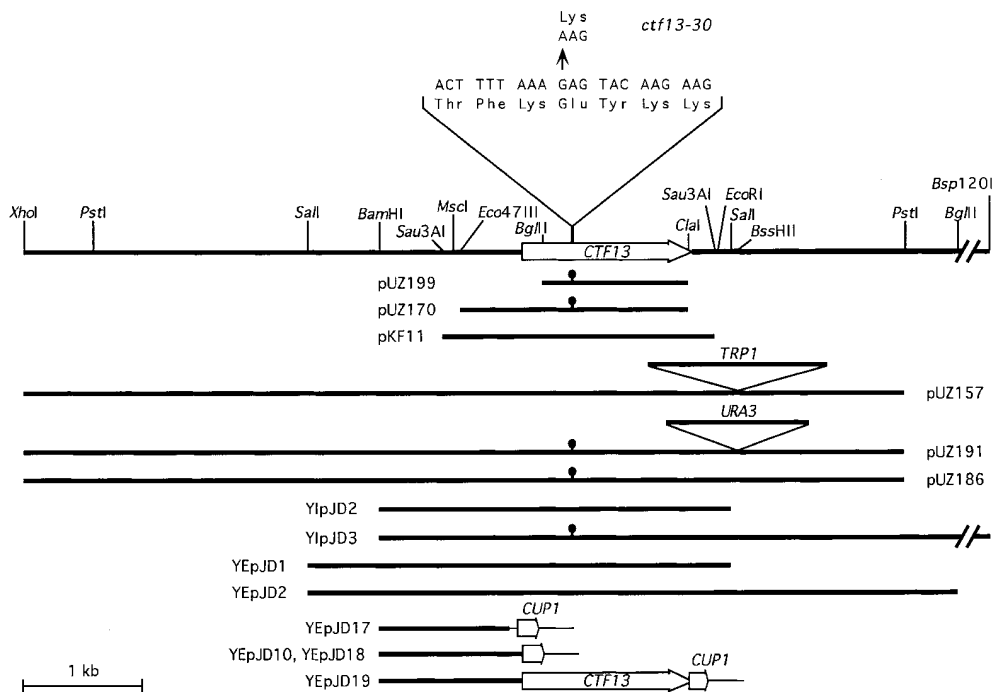
Figure 1.—Map of the *CTF13* region. The *CTF13* ORF is shown by the open arrow pointing in the direction of transcription. The locations of restriction sites mentioned in the text are shown on the same line. The distance indicated by the break in the line at the right end is 2.8 kb. The wild-type nucleotide and amino acid sequence surrounding the *ctf13-30* 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 *ctf13-30* 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).

abundance of U6 RNA, it was necessary to adjust the specific activity of riboprobe 3 downward by dilution of $[\alpha\text{-}^{32}P]CTP$ with cold CTP during synthesis. A cocktail containing RNase A and RNase T_1 was added to each sample. The protected fragments were denatured in 80% formamide loading buffer at 95° for 5 min and fractionated on a 5% polyacrylamide/8 m urea gel. To measure mRNA half-lives, transcription was terminated by temperature-shift from 25° to 36° in strains carrying *rpb1-1*, which codes for a Ts RNA polymerase II mutant (Parker *et al.* 1991). RNA was extracted from cell samples taken at time intervals following temperature-shift as indicated. Decay of mRNA was followed by RNase protection or Northern blotting.

Antisense oligonucleotide-directed RNase H cleavage was performed as follows. Oligonucleotide (1 pmol) was added to 8–10 μg of total yeast RNA in buffer H (40 mm Tris-HCl, pH 7.7, 4 mm $MgCl_2$, 1 mm dithiothreitol, and 30 μg/ml bovine serum albumin). The sequence of oligonucleotides used is as follows: (1) 5′-GTA GGG ATG TGC TCC GC-3′, (2) 5′-AGC ATT TGT CTA CTA GTT GTG G-3′, (3) 5′-TCA TGA TTG GTT TAT CAG ACA G-3′, (4) 5′-AGT CTT CTC CAG TCA TCG TG-3′, and (5) 5′-TCA CCA CCC AAT TTC GGA AAG-3′. Samples were denatured for 3 min at 75° and were step-cooled in 5° decrements every 3 min to 30° using a thermocycler. RNase H (Promega) was added (0.1 units) and samples were incubated at 30° for 30 min. Samples were analyzed by Northern blotting.

Data quantification: Molecular Dynamics (Sunnyvale, CA) ImageQuant software (version 4.1) was used to quantify data from gels or blots digitally recorded on a Molecular Dynamics PhosphorImager (model 425). Bands of interest were surrounded with an object border to determine the enclosed relative signal. For RNase protection results, the values from

protected riboprobe 1 were normalized using the corresponding values from protected riboprobe 3 as a means to control for experimental variation. For blotting experiments, the values for bands of interest were similarly normalized to control band values as described in results.

The relative abundance of *CTF13* mRNA and the comparative difference in *CTF13* and *ctf13-30* mRNA levels were determined. The specific activity of riboprobes 1 and 3 was adjusted for the change in size and $[^{32}P]$cytidine content expected to result from mRNA-protected RNase digestion and converted to molar activities [(counts per minute) per micromole]. The ratio of the molar activity of the two RNA probes was used to correct the ratio of *CTF13* or *ctf13-30* mRNA to U6 RNA derived from RNase protection experiments (see results). The resulting ratios for *CTF13* and *ctf13-30* 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.

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 *Bgl*II-*Not*I fragment derived from plasmid pUZ157, containing both *CTF13* and *TRP1* from *Bgl*II to *Pst*I, 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 *Bgl*II-*Pst*I fragment supplies *CTF13* function. Another experiment was performed in which a *Bgl*II-*Not*I fragment derived from plasmid pUZ191, containing both *ctf13-30* and *URA3* from *Bgl*II to *Pst*I, 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-fluororotic 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-sectoring assay described previously (Hieter *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− 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 *Bss*HII 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

TABLE 1

Yeast strains

Strain	Genotype
YK33	*MAT*α *ctf13-30 ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 [CFIII (CEN3.L) SUP11 URA3]*
YK35	*MAT*α *ctf13-30 ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 [CFVII (RAD2.d) SUP11 URA3]*
YK41[a]	*MAT*a *ctf13-30 ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ1 [CFIII (CEN3.L) SUP11 URA3]*
YK113	*MAT*α *ura3-52 lys2-801 ade2-101 leu2-Δ1 his3-Δ200 trp1-Δ1 ctf13-30::HIS3 [CFVII (RAD2.d) SUP11 TRP1] [2μ CTF13 URA3]*
YJP101	*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 CTF13::URA3 [YAC12 HIS3 ADE2 LYS2]*
YJP102	*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 CTF13::URA3 [YAC12 HIS3 ADE2 LYS2]*
YJP108	*MAT*α *ctf13-30 ura3-52 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 [CFIII (CEN3.L) SUP11 URA3]*
YJP112	*MAT*a *ctf13-30 ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ1 upf1-Δ2::URA3*
YJP113	*MAT*α *ctf13-30 ura3-52 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 upf2-Δ1::HIS3*
JDY1	*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ1 ctf13-30::YIpJD2*
JDY3	*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ1*
JDY5	*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ1 upf1-Δ2::URA3*
JDY6	*MAT*a *ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ1 ctf13-30::YIpJD3*
JDY7	*MAT*a *ura3-52 trp1-Δ1 leu2-3,112 upf1-Δ2::URA3 rpb1-1*
JDY12	*MAT*α *ura3-52 leu2-3,112 trp1-289 his3-11,15 cup1Δ*
JDY17	*MAT*α *ura3-52 leu2-3,112 trp1-289 his3-11,15 cup1Δ upf1-Δ4::HIS3*
JDY21	*MAT*a *ura3-52 leu2-3,112 trp rpb1-1 upf1-Δ4::HIS3*
JDY22	*MAT*α *ura3-52 leu2-3,112 trp1 his3-11,15 rpb1-1 cup1Δ upf1-Δ4::HIS3*
JDY29	*MAT*a *ura3-52 leu2-Δ1,112 trp1 his3-11,15 cup1Δ upf1-Δ4::HIS3*

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

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 (Riles *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* (*NMD2*), 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 *Apa*I site in the

UPF1 ORF and a *Bsp*EI 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 Culbertson 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 (materials and methods) were transformed with each of the three plasmids. The transformants were assayed for growth at 37°. 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° regardless of the presence of any of the three plasmids. Pairwise crosses revealed that all eight suppressor mutations failed to
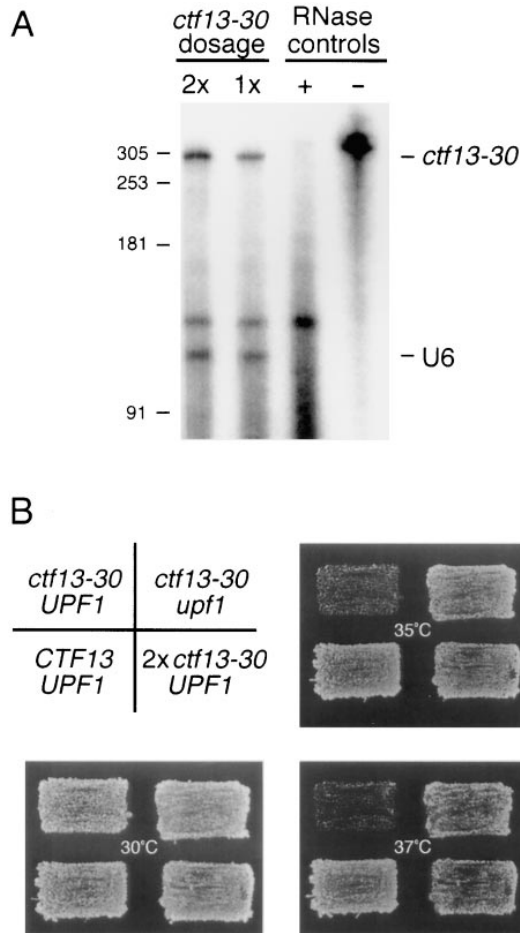
Figure 2.—Relationship between mRNA abundance and growth. (A) mRNA abundance was determined by an RNase protection assay (materials and methods). The PhosphorImage shows representative RNase-protected bands from riboprobe 1 and riboprobe 3 using total RNA from haploid strains JDY6 ($2\times$ *ctf13-30* dosage) and YK41-CF$^-$ ($1\times$ *ctf13-30* dosage). Control RNA (sheared *Torula* RNA) was incubated with ($+$) or without ($-$) RNase treatment. The protected RNAs were resolved on a 5% polyacrylamide/8 m urea gel. The sizes of the protected RNAs were estimated by comparison with RNA molecular weight standards (left). The expected sizes for protected riboprobes 1 and 3 are 298 nt and 112 nt, respectively. (B) Relative growth rates were determined by replica plating patches of cells on synthetic complete medium. Plates were incubated at the indicated temperatures and photographed 2 days later. The grid in the upper left indicates the position and the relevant genotypes of isogenic strains used, which were YK41-CF$^-$ (*ctf13-30 UPF1*), YJP112 (*ctf13-30 upf1-Δ2*), JDY3 (*CTF13 UPF1*), and JDY6 ($2\times$ *ctf13-30 UPF1*).

recombine and are therefore likely to be located in a single gene, which we have named *ICK1* (*I*nteraction with *C*tf13p or *K*inetochore). Additional crosses with one representative *ick1* suppressor showed that the *ick1* 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 muta-

tions 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 non-sense-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. By 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 *upf1-Δ2* (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 *ick1* 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 analyzed by RNase 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 *ick1* 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 *ick1* 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-

TABLE 2

Plasmids

Plasmid	Vector	Yeast genes[k]
pRS315[a]	—	*LEU2, CEN6, ARS4*
pRS316[a]	—	*URA3, CEN6, ARS4*
pUZ170[b]	pRS314[a]	*TRP1, CEN6, ARS4, ctf13-30(Eco47III-ClaI)*
pUZ199[b]	pRS314[a]	*TRP1, CEN6, ARS4, ctf13-30(BglII-ClaI)*
pUZ157[b]	pRS314[a]	*TRP1, CEN6, ARS4, CTF13(XhoI-PstI):TRP1*
pUZ191[b]	pRS314[a]	*TRP1, CEN6, ARS4, ctf13-30(XhoI-PstI):URA3*
pUZ186[b]	pRS315	*LEU2, CEN6, ARS4, ctf13-30(XhoI-PstI)*
pRS315UPF1[c]	pRS315	*LEU2, CEN6, ARS4, UPF1(BamHI-EcoRI)*
pRS316UPF1[c]	pRS316	*URA3, CEN6, ARS4, UPF1(BamHI-EcoRI)*
pUZ178[d]	pRS316	*URA3, CEN6, ARS4, UPF2(XbaI-SacI)*
pLS17[d]	pRS316	*URA3, CEN6, ARS4, UPF2(SpeI-KpnI)*
pCTF14[e]	YPCp50[e]	*LEU2, CEN4, ARS1, CTF14*
YIpJD2[b]	pRS305[a]	*LEU2, CTF13(BamHI-SalI)*
YIpJD3[b]	pRS305[a]	*LEU2, ctf13-30(BamHI-Bsp1201)*
YEpJD1[b]	YEp351[f]	*LEU2, 2μ ori, CTF13(SalI-SalI)*
YEpJD2[b]	YEp351[f]	*LEU2, 2μ ori, CTF13(SalI-BglII)*
YEpJD10[h,g]	pRS426[h]	*URA3, 2μ ori, CTF13(BamHI-nt −1):CUP1 (nt+1=KpnI)*
YEpJD17[b,g]	YEp351[f]	*LEU2, 2μ ori, CTF13(BamHI-nt−110):CUP1 (nt-65-KpnI)*
YEpJD18[b,g]	YEp351[f]	*LEU2 2μ ori, CTF13(BamHI-nt−1):CUP1 (nt+1-KpnI)*
YEpJD19[b,g]	YEp351[f]	*LEU2, 2μ ori, CTF13(BamHI-nt+1403): CUP1(nt+18-KpnI)*
pPL65[i]	pBR322	*upf1-Δ2::URA3*
pUPF1-Δ4[b]	pBR322	*upf1-Δ4::HIS3*
pKF11[j]	pRS314	*TRP1, CEN6, ARS4 CTF13(2.2-kb Sau3AI)*

[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] Hill *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] Doheny *et al.* (1993).

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

pressor mutations in *ICK1* have no effect on the accumulation of *ctf13-30* mRNA.

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

TABLE 3

Extragenic suppressors of *ctf13-30*

	Parental strains			
Gene	YK41	YK35	YJP108	Total
UPF1	19	8	11	38
UPF2	17	2	5	24
UPF3	5	2	0	7
ICK1	6	0	2	8
Total	47	12	18	77

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 ³²P-labeled riboprobes 1 and 3 using RNA extracted from strain YK41-CF⁻ (*ctf13-30 UPF1*) and YJP112 (*ctf13-30 upf1-Δ2*) (Figure 4). The amount of riboprobe 1 remaining following RNase digestion was 2.1 ± 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.

To assess the effect of complete loss of *UPF1* function on suppression of *ctf13-30*, the growth of strain YJP112 (*upf1-Δ2*, 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° and 37°. YJP112, JDY6, and JDY3 grew at both
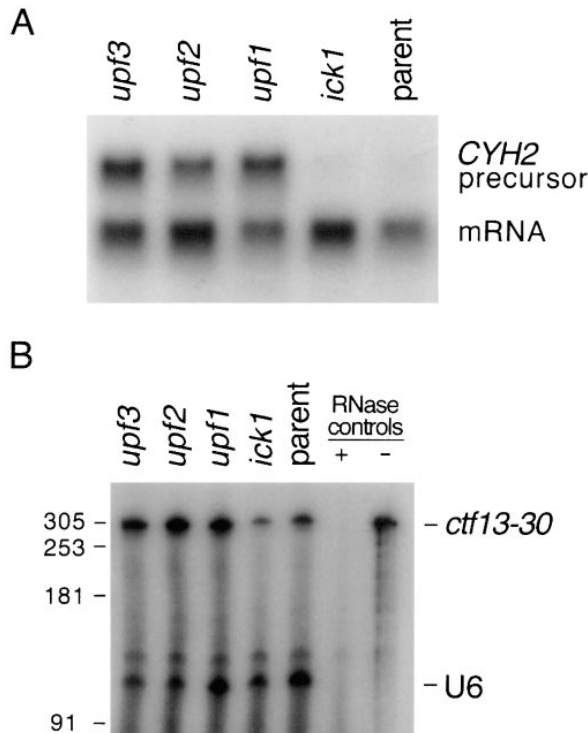
A

B

Figure 3.—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 *ick1*) and from strain YK41-CF⁻ (parent). The RNAs were resolved on a 1% agarose/16.2% formaldehyde gel. (B) The autoradiogram shows representative RNase-protected 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 riboprobe 1 and riboprobe 3 were estimated by comparison with RNA molecular weight standards (left). The RNAs were resolved on a 5% polyacrylamide/8 m urea gel.

temperatures, but the growth of JDY3 was discernably more robust (Figure 2). This suggests that the increased accumulation of *ctf13-30* mRNA resulting from the presence of *upf1-Δ2* leads to suppression of the growth defect conferred by *ctf13-30.* However, the growth rate due to suppression is still less than the growth rate of a *CTF13* strain.

Using the colony-sectoring assay (Hieter *et al.* 1985), mitotic chromosome stability was examined in *ctf13-30* strains carrying a suppressor mutation in *UPF1* that appeared by growth tests to confer complete or nearly complete loss of *UPF1* function. We found that this suppressor mutation caused a significant reduction in the occurrence of red sectors (Figure 5). Similar results were found using a suppressor mutation in *UPF2*. These results indicate that impaired function of *UPF1* or *UPF2* restores stable propagation of a chromosome fragment
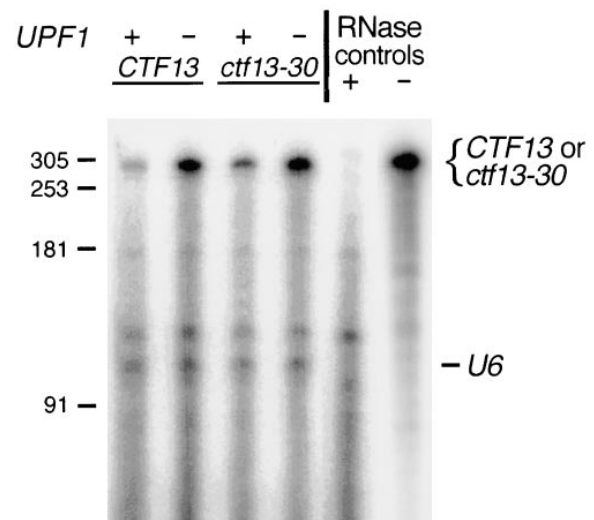

Figure 4.—Effect of *UPF1* disruption on the accumulation of *CTF13* and *ctf13-30* mRNA. The PhosphorImage shows representative results of RNase protection (see Figure 2) using total RNA from isogenic strains JDY3 (*CTF13 UPF1*), JDY5 (*CTF13 upf1*), YK41-CF⁻ (*ctf13-30 UPF1*), and YJP112 (*ctf13-30 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 m urea gel.

that is otherwise unstably propagated due to the *ctf13-30* mutation.

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 ± 0.7-fold higher ($n = 8$) when the RNA used was from strain JDY5 (*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 3 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 $2–4 \times 10^4$-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).
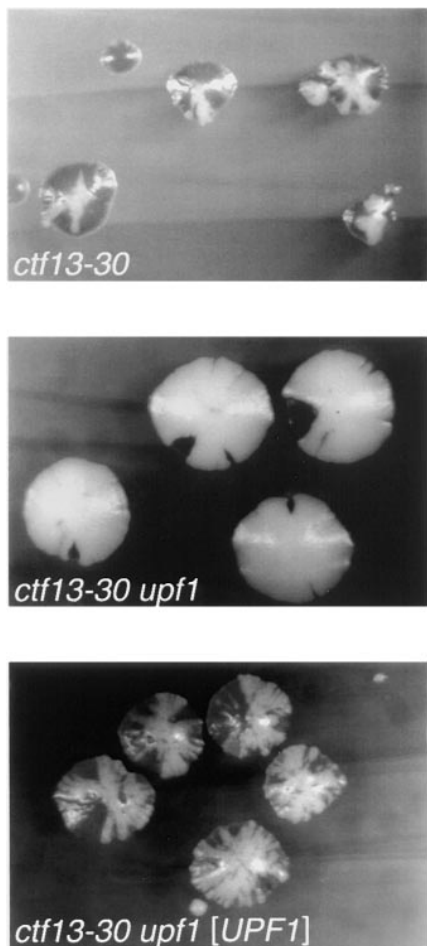
Figure 5.—Suppressors in *UPF* genes restore chromosome stability in strains carrying *ctf13-30*. A colony-sectoring assay was used to monitor loss of a centromere-containing chromosome fragment carrying the *SUP11* ochre suppressor as described previously (Hieter *et al.* 1985). Strains carrying the ochre mutation *ade2-101* produce a red colony color, whereas *SUP11* suppresses *ade2-101*, resulting in a white colony color. The appearance of red sectors provides a measure of the rate of chromosome loss. The sectoring phenotype and corresponding relative genotype is shown for the strains used, which are YK41 (*ctf13-30*), a YK41 derivative carrying a suppressor mutation in the *UPF1* gene (*ctf13-30 upf1*), and the same YK41 derivative following transformation with a plasmid carrying a wild-type *UPF1* gene (*ctf13-30 upf1* [*UPF1*]). Similar results were obtained for a suppressor mutation in *UPF2* (data not shown).

The *CTF13* mRNA has a long extension at the 3′ end: To determine the size of the *CTF13* mRNA hybridizing to riboprobe 1 in RNase 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 (Doheny *et al.* 1993). To resolve this discrepancy, we sought to overexpress the authentic *CTF13* mRNA using multicopy 2μ plasmids. *CTF13* multicopy 2μ plasmids YEpJD1 and YEpJD2 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 YEpJD1 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 YEpJD1. To test this possibility, we used plasmid YEpJD2, 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 YEpJD2 (Figure 6A). This indicates that the authentic *CTF13* transcription terminator sequences are present in YEpJD2 and lacking in YEpJD1. 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 RNase H cleavage (Figure 6B). Total RNA from JDY17 carrying YEpJD2 was hybridized to a series of oligonucleotides and treated with RNase 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
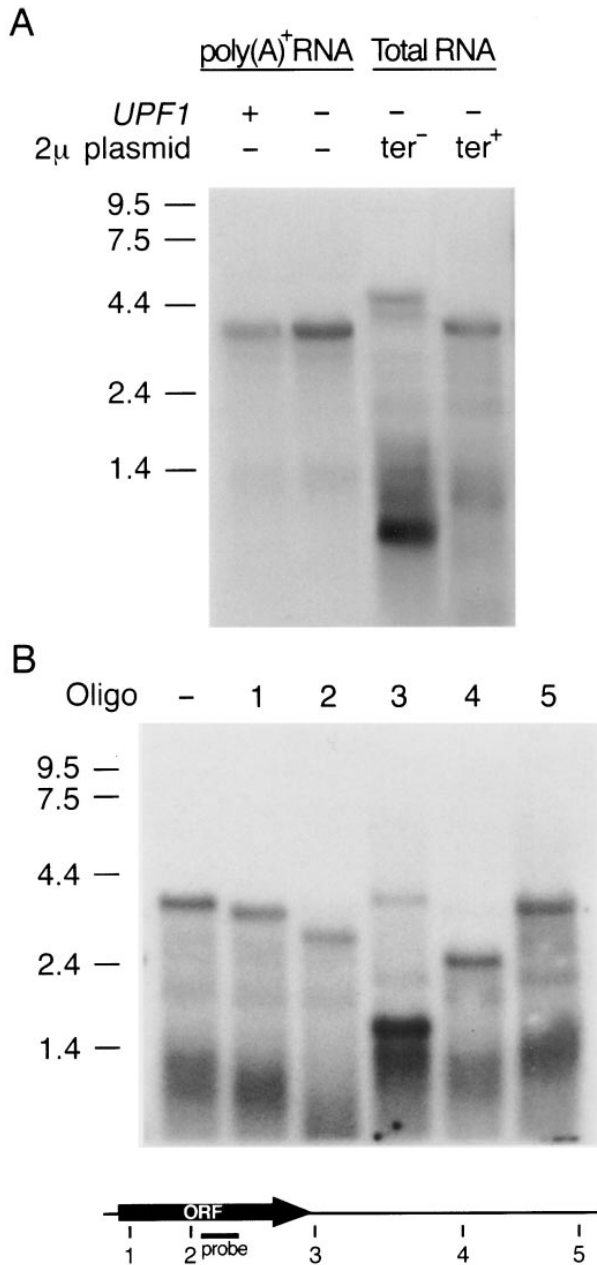
Figure 6.—Detection and mapping of *CTF13* mRNA by Northern blotting. (A) The autoradiogram shows the result of a Northern blot of 10 μg of poly(A)$^+$ RNA from strains without plasmids that are *UPF1* wild type (+) (JDY12) or *upf1* null (−) (JDY17) and 10 μg of total RNA from strains carrying either YEpJD1 (ter$^-$) or YEpJD2 (ter$^+$) that are *upf1* null (−) (JDY17) hybridized with riboprobe 1 (materials and methods). RNA samples were denatured by glyoxal/DMSO treatment prior to being resolved on a 1% agarose gel. (B) Total RNA (8 μg) from JDY17 carrying YEpJD2 was hybridized separately to each of five oligonucleotides and subjected to digestion with RNase H. The left lane (−) was mock treated with no oligonucleotide. RNA samples were denatured and fractionated as in A and, after transfer, hybridized with riboprobe 1. The positions of the oligonucleotides (numbers) and riboprobe 1 (probe) relative to the *CTF13* ORF are depicted in the schematic below, and the lane numbers correspond to the numbers assigned to each oligonucleotide.

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° to 36°, 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° to 36°, 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[pRS315] compared to JDY21[pRS315UPF1] (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* promotor-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 promotor activity of various DNA fragments from the 5′ side of *CTF13* (Figure 1; materials and methods).

All three gene fusions include DNA from the *Bam*HI site 1.2 kb 5′ of the *CTF13* translation start site up to the
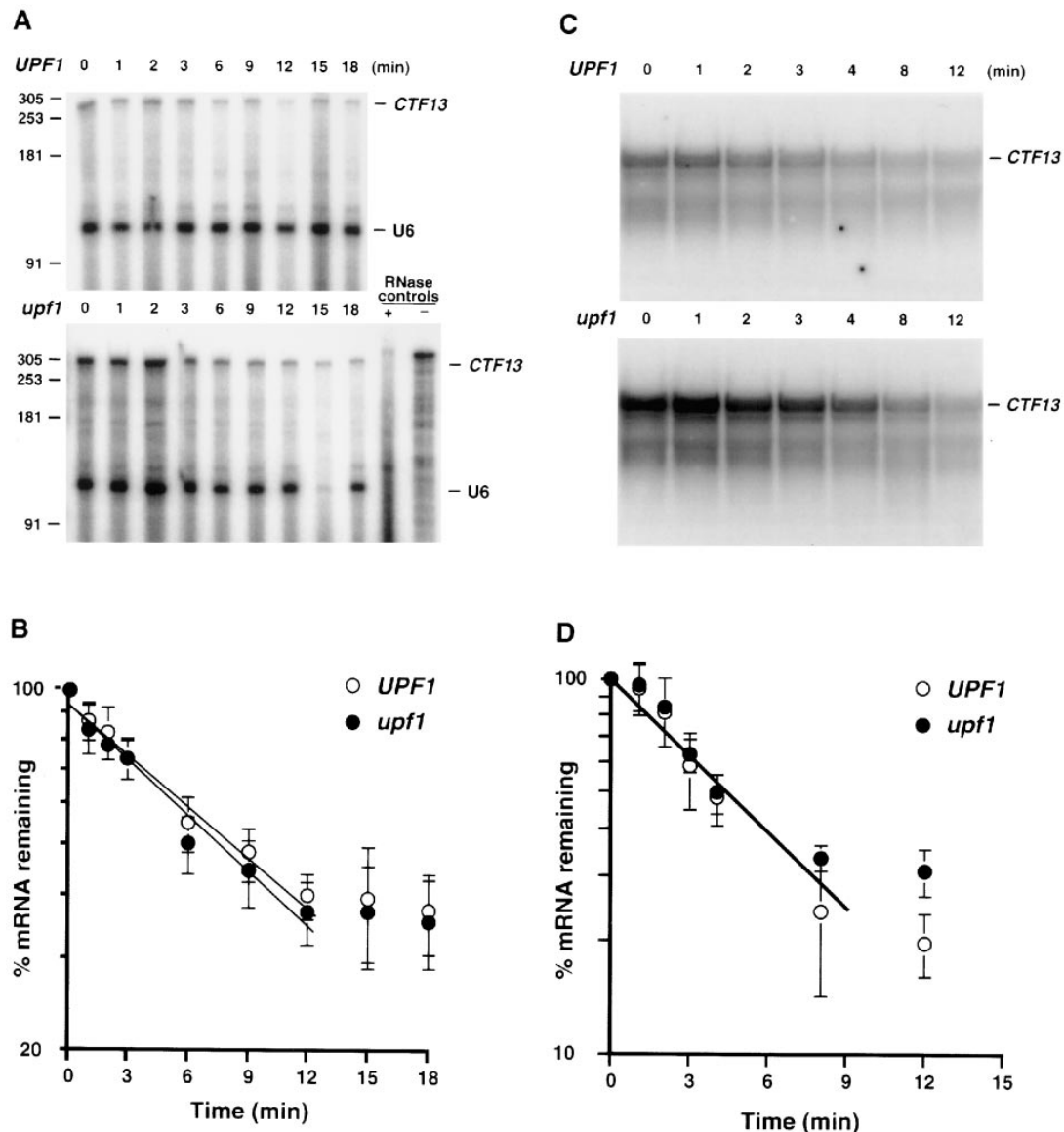
Figure 7.—CTF13 mRNA half-life in *UPF1* wild-type and *upf1* null strains. The *CTF13* mRNA half-life was determined by RNase protection (A and B) or by Northern blot (C and D) analysis of RNA extracted from samples collected at time intervals following termination of transcription as shown using riboprobe 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
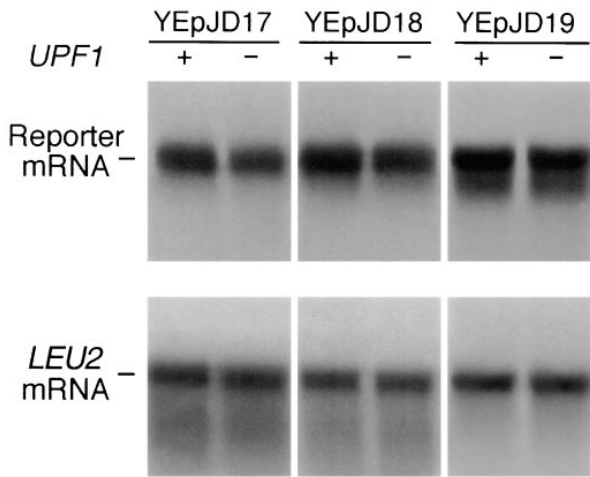
Figure 8.—Gene fusion reporter mRNA accumulation in *UPF1* wild-type and *upf1* null strains. The autoradiogram shows representative results of a Northern blot of total RNA extracted from JDY29 (*upf1-Δ4*) transformed with pRS316UPF1 (+) or pRS316 (−) and the multicopy plasmids, each of which carries a different *CTF13-CUP1* gene fusion. (Top) RNA from strains carrying YEpJD17 or YEpJD18 was hybridized to riboprobe 2 and RNA from strains carrying YEpJD19 was hybridized to riboprobe 1. (Bottom) *LEU2* mRNA expressed from the multicopy plasmids was detected. The same blots (after being stripped) were hybridized to DNA probes derived from a 600 nt *Cla*I-*Eco*RI *LEU2* fragment. Since this fragment is missing from the *leu2-Δ1* allele carried in JDY29, the signal represents only mRNA expressed from the plasmid. RNA samples were denatured by glyoxal/DMSO treatment prior to being resolved on a 1% agarose gel.

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 *CTF13* promoter.

Plasmids carrying each of the gene fusions were transformed into the *upf1* mutant strain JDY29 (Table 1). Isogenic strains were made by transforming these strains with pRS316 or pRS136UPF1, which carries *UPF1* (Table 2). 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 trans-

formed 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 possible. We found that a haploid strain carrying two copies of the *ctf13-30* allele grows at the restrictive temperature, suggesting that mutations leading to an increase in gene expression might be represented among suppressors. 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 *ick1* 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 (Doheny *et al.* 1993). 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 G$_2$/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 (Spencer and Hieter 1992; Doheny *et al.* 1993). As discussed above, the increase in *ctf13-30* mRNA accumulation relative to wild type at the permissive temperature might result from mitotic delay, which could be imposed by checkpoint surveillance to promote kinetochore assembly and growth. The level of *ctf13-30* mRNA at the restrictive temperature presumably fails to promote assembly of

functional kinetochores, resulting in checkpoint signaling of a G$_2$/M phase growth arrest. The additional increase in *ctf13-30* mRNA accumulation resulting from gene duplication or *upf* suppressor mutations might promote assembly of functional kinetochores and allow bypass of the G$_2$/M phase growth arrest, thereby alleviating the Ts phenotype.

Suppressor mutations in the *ICK1* gene cause no change in the *ctf13-30* mRNA levels. Mutations in *ick1* must therefore suppress the *ctf13-30* mutation by an alternative, post-transcriptional mechanism that compensates for the altered function or stability of ctf13-30p. In light of the finding that Ctf13p is subject to ubiquitin-mediated degradation (Kaplan *et al.* 1997), it is tempting to speculate that *ICK1* may encode a factor required for ubiquitination or degradation of Ctf13p. Alternatively, suppressor mutations in *ICK1* may compensate for the altered function or stability of ctf13-30p by some other mechanism.

The role of *UPF*-mediated mRNA decay in *CTF13* expression: In this article we have focused on suppressors that restore kinetochore function through a change in the accumulation of *ctf13-30* mRNA. We found that suppressors in *UPF1*, *UPF2*, and *UPF3* cause an increase in accumulation of *ctf13-30* mRNA sufficient in magnitude to confer growth at the restrictive temperature as judged by comparison with the growth effects of a *ctf13-30* gene duplication. In addition to the increased *ctf13-30* mRNA abundance in *upf* suppressor and *upf1* null strains, we observed a similar increase in accumulation of wild-type *CTF13* mRNA when the *UPF1* gene is inactivated. Therefore, the *ctf13-30* mutation cannot itself cause the *UPF*-mediated effect on *ctf13-30* mRNA accumulation. Our results suggest that *UPF*-mediated mRNA decay is part of the natural circuitry regulating *CTF13* gene expression.

Three lines of evidence support this view. First, the *UPF* genes encode products that perform related functions in accelerated decay of nonsense mutant mRNAs and pre-mRNAs (Leeds *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 impairment of the *UPF*-mediated RNA 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-

jority 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 Peltz 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* (Losson *et al.* 1983). Consistent with this idea, we have observed that the *PPR1* mRNA accumulates to a higher level in *upf* mutant strains (M. Lelivelt and M. R. Culbertson, 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 promotor-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 a nonabundant 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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