EUKARYOTIC CELL, Feb. 2003, p. 134–142 Vol. 2, No. 1
1535-9778/03/$08.00+0 DOI: 10.1128/EC.2.1.134–142.2003

mRNAs Encoding Telomerase Components and Regulators Are Controlled by *UPF* Genes in *Saccharomyces cerevisiae*

Jeffrey N. Dahlseid,[1]† Jodi Lew-Smith,[2]‡ Michael J. Lelivelt,[3] Shinichiro Enomoto,[2] Amanda Ford,[3] Michelle Desruisseaux,[2] Mark McClellan,[2] Neal Lue,[4] Michael R. Culbertson,[3] and Judith Berman[2,5]*

Department of Chemistry, St. Olaf College, Northfield, Minnesota 55057[1]; Department of Genetics, Cell Biology and Development[2] and Department of Microbiology,[5] University of Minnesota, Minneapolis, Minnesota 55455; Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706[3]; and Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, New York 10021[4]

Received 13 August 2002/Accepted 31 October 2002

Telomeres, the chromosome ends, are maintained by a balance of activities that erode and replace the terminal DNA sequences. Furthermore, telomere-proximal genes are often silenced in an epigenetic manner. In *Saccharomyces cerevisiae*, average telomere length and telomeric silencing are reduced by loss of function of *UPF* genes required in the nonsense-mediated mRNA decay (NMD) pathway. Because NMD controls the mRNA levels of several hundred wild-type genes, we tested the hypothesis that NMD affects the expression of genes important for telomere functions. In *upf* mutants, high-density oligonucleotide microarrays and Northern blots revealed that the levels of mRNAs were increased for genes encoding the telomerase catalytic subunit (Est2p), in vivo regulators of telomerase (Est1p, Est3p, Stn1p, and Ten1p), and proteins that affect telomeric chromatin structure (Sas2p and Orc5p). We investigated whether overexpressing these genes could mimic the telomere length and telomeric silencing phenotypes seen previously in *upf* mutant strains. Increased dosage of *STN1*, especially in combination with increased dosage of *TEN1*, resulted in reduced telomere length that was indistinguishable from that in *upf* mutants. Increased levels of *STN1* together with *EST2* resulted in reduced telomeric silencing like that of *upf* mutants. The half-life of *STN1* mRNA was not altered in *upf* mutant strains, suggesting that an NMD-controlled transcription factor regulates the levels of *STN1* mRNA. Together, these results suggest that NMD maintains the balance of gene products that control telomere length and telomeric silencing primarily by maintaining appropriate levels of *STN1*, *TEN1*, and *EST2* mRNA.

Telomeres, the ends of linear chromosomes, are important for chromosome integrity and are maintained by telomerase, a reverse transcriptase-like enzyme that includes an integral RNA template. The catalytic components of *Saccharomyces cerevisiae* telomerase (*TLC1* RNA and Est2p), as well as gene products required for telomerase activity in vivo (e.g., Est1p, Est3p, Cdc13/Est4p, Ku70/80, Mec1p, MRX, Rap1p, Stn1p, Tel1p, and Ten1p), have been identified (reviewed in reference 12). However, mechanisms that regulate the expression and activity of telomerase components and modulators have not been explored.

The nonsense-mediated mRNA decay (NMD) pathway accelerates the degradation of mRNAs that prematurely terminate translation due to nonsense mutations, frameshifts, or translation of alternate open reading frames (ORFs) within the mRNA (21, 37). In *S. cerevisiae*, the products of *UPF1*, *UPF2*, and *UPF3* are required for NMD and provide a surveillance function to lower the abundance of potentially deleterious protein fragments by degrading mRNAs that cannot be translated full length (42). However, the only known growth phenotype of *upf* mutants is deficient respiration (1). Interestingly, NMD also controls the expression of some wild-type genes. By using high-density oligonucleotide arrays (HDOAs), several hundred wild-type *S. cerevisiae* mRNAs with either increased or decreased steady-state levels in *upf* mutants were identified (23). NMD directly regulates the level of wild-type mRNAs for some genes, such as *SPT10* and *CPA1*, through accelerated degradation triggered by translation of alternate ORFs within the mRNA (46, 50). Given the large number of genes controlled by NMD, including transcription factors such as Ppr1p (29, 45) and Ino4p (23), many wild-type mRNAs are likely to change in abundance as an indirect consequence of changes in the abundance of transcriptional regulators (23).

Previously it has been found that mutations in *UPF1*, *UPF2*, or *UPF3* reduced telomere length and silencing of a telomere-adjacent reporter gene (25). It was hypothesized that NMD regulates telomeres by altering the levels of specific wild-type mRNAs important for telomere functions. To identify genes important for the telomere-related phenotypes of *upf* mutants, we screened the HDOA data of Lelivelt et al. (23) for *S. cerevisiae* genes thought to be important for telomere functions. Here we report that mRNAs encoding the catalytic subunit of telomerase, regulators of telomerase activity, and proteins that affect telomeric silencing are all controlled by NMD. Furthermore, extra copies of *EST2*, *STN1*, and *TEN1* were

* Corresponding author. Mailing address: Department of Genetics, Cell Biology, and Development, University of Minnesota, 6-160 Jackson Hall, 321 Church St. SE, Minneapolis, MN 55455. Phone: (612) 625-1971. Fax: (612) 625-6140. E-mail: judith@cbs.umn.edu.

† Present address: Departments of Biology and Chemistry, Gustavus Adolphus College, Saint Peter, MN 56082.

‡ Present address: Incyte Genomics, Inc., Proteome Division, Beverly, MA 01915.

TABLE 1. Expression of selected genes with possible telomere-related functions in *upf* mutant strains relative to that in *UPF* strains

ORF	Gene name	CKI[a]	AFC[b]	ORF	Gene name	CKI[a]	AFC[b]
YLR010C	*TEN1*	1.00	3.3	YDR225W	*HTA1*	0.00	0.90
YMR127C	*SAS2*	0.88	3.20	YDR224C	*HTB1*	0.00	0.86
YNL261W	*ORC5*	0.67	1.75	YJL076W	*NET1*	0.00	1.05
YDR082W	*STN1*	0.59	2.55	YML065W	*ORC1*	0.00	1.06
YBR195C	*MSI1*/*CAC3*	0.56	2.29	YLL004W	*ORC3*	0.00	1.15
YLR318W	*EST2*	0.56	2.45	YKL113C	*RAD27*	0.00	0.94
YLR233C	*EST1*	0.53	2.25	YML032C	*RAD52*	0.00	0.88
YCL011C	*RLF6*	0.44	1.30	YDR217C	*RAD9*	0.00	0.20
YMR106C	*HDF2*	0.25	1.40	YOR217W	*RFC1*	0.00	0.99
YML061C	*PIF1*	0.22	2.05	YBR275C	*RIF1*	0.00	0.97
YLR453C	*RIF2*	0.22	1.90	YBL092W	*RPL32*	0.00	0.93
YMR284W	*HDF1*	0.19	1.53	YDR227W	*SIR4*	0.00	1.33
YPR018W	*CAC1*/*RLF2*	0.13	0.25	YLR234W	*TOP3*	0.00	0.00
YPL001W	*HAT1*	0.13	0.98	YER151C	*UBP3*	0.00	1.41
YLR223C	*IFH1*	0.13	0.98	YDR440W	*DOT1*	−0.03	0.87
YNL250W	*RAD50*	0.13	7.03	YBL008W	*HIR1*	−0.03	1.37
YPL128C	*TBF1*	0.13	3.70	YHR013C	*ARD1*	−0.06	1.13
YGR099W	*TEL2*	0.13	1.02	YJL115W	*ASF1*	−0.06	1.15
YML102W	*CAC2*	0.09	1.06	YDL160C	*DHH1*	−0.06	1.22
YMR224C	*MRE11*	0.09	1.32	YNL021W	*HDA1*	−0.06	0.00
YPR162C	*ORC4*	0.09	1.46	YBR009C	*HHF1*	−0.06	0.93
YPL153C	*RAD53*	0.09	4.60	YBR060C	*ORC2*	−0.06	1.51
YKR101W	*SIR1*	0.09	1.42	YBL052C	*SAS3*	−0.06	1.03
YOR351C	*MEK1*	0.07	1.07	YOR230W	*WTM1*	−0.06	0.82
YOL051W	*GAL11*	0.06	1.13	YNL031C	*HHT2*	−0.07	0.90
YEL056W	*HAT2*	0.06	1.13	YJR138W	*HIR3*	−0.09	1.66
YOR025W	*HST3*	0.06	1.33	YIL010W	*DOT5*	−0.13	1.00
YDR191W	*HST4*	0.06	1.26	YNL216W	*RAP1*	−0.13	0.77
YNL330C	*RPD3*	0.06	1.03	YLR442C	*SIR3*	−0.13	0.96
YDR369C	*XRS2*	0.06	1.03	YPL139C	*WTM3*	−0.13	0.91
YNL102W	*CDC17*	0.03	1.15	YDL042C	*SIR2*	−0.16	0.85
YOR038C	*HIR2*	0.03	0.98	YNL030W	*HHF2*	−0.19	0.71
YGL058W	*RAD6*	0.03	1.06	YBR010W	*HHT1*	−0.19	0.88
YBL088C	*TEL1*	0.03	1.12	YBL002W	*HTB2*	−0.19	0.80
YOR229W	*WTM2*	0.03	0.87	YDL040C	*NAT1*	−0.19	1.08
YDL220C	*CDC13*	0.00	1.14	YHR119W	*SET1*	−0.19	1.01
YOR217W	*CDC44*	0.00	0.99	YGL173C	*KEM1*	−0.22	0.84
YER088C	*DOT6*	0.00	1.08	YBL003C	*HTA2*	−0.25	0.81

[a] CKI (combined knockout index) was defined by Lelivelt and Culbertson (23). For each mRNA, numerical weights were assigned to GeneChip difference calls for four trials for each of the four *upf* mutant strains compared to four trials for an isogenic *UPF* strain as follows: increased signal (+2), statistically marginal increase (+1), no change (0), statistically marginal decrease (−1), and decrease (−2). The numerical values of the difference calls were summed across all trials and then divided by the maximum potential score to yield the CKI score. Scores of ≥0.5 indicate that the given mRNA was increased in abundance with relative consistency from trial to trial, whereas scores of ≤−0.5 indicate that a given mRNA was decreased in abundance with similar relative consistency.

[b] AFC (average fold change) was measured independently of the CKI score. The AFC is the average increase or decrease in mRNA abundance from 16 trials with *upf* mutants compared with four trials with *UPF* strains. AFCs for some mRNAs with CKI scores between 0.5 and −0.5 are not reliable because the CKI score indicates poor consistency of calls across trials.

sufficient to mimic the telomeric silencing and telomere length phenotypes of *upf* mutants.

MATERIALS AND METHODS

Yeast strains, plasmids, and growth conditions. The yeast strain used for HDOA studies was LRSy307 (*MAT***a** *his3-11,15 trp1-*Δ*1 leu2-*Δ*1 ura3-52 upf1*::*ura3*[5-fluoroorotic acid {FOA} resistant] *upf2*::*HIS3 upf3*::*TRP1*) transformed with *UPF* plasmids as described in Lelivelt et al. (23). Data from the HDOA experiments can be found at the following website: http://144.92.19.47/default.htm. Northern analysis was done using the same strains or with YJB276 (*MAT***a** *leu2-3,112 ura3-52 trp1-289 his3*Δ *ade2*Δ), YJB2763 (*MAT***a** *leu2-3,112 ura3-52 trp1-289 his3*Δ *ade2*Δ *nmd2*::*HIS3 adh4*::*URA3-TEL*), or YJB487 (*MAT***a** *leu2-3,112 ura3-52 his3*Δ *ade2*Δ *adh4*::*URA3-TEL*) transformed with either pRS315-NMD2 or pRS315 (25). Telomeric silencing assays were performed as described previously (16) using YJB487 transformed with the indicated plasmids and strain YJB539 (*MAT***a** *leu2-3,112 ura3-52 his3*Δ *ade2*Δ *rlf4-1 adh4*::*URA3-TEL*) carrying a *upf2*/*nmd2* mutation as a control strain. Telomere length assays, telomerase assays, and *TLC1* RNase protection experiments were performed using strain YJB209 (*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1*, *ura3-1*) transformed with appropriate plasmids and crossed with strain YJB195 (*MAT***a** *ura3-1 ade2-1 his3-11,15 leu2-3,112 can1-100 trp1-1*) transformed with YEplac181, p2μm-*STN1a*, and p2μm-*EST2*, respectively, as described below and in the figure legends. RNA half-life experiments were conducted using strain AAY333 (*MAT*α *ADE2 ura3 his3-11,15 trp1-1 leu2-3,112 rpb1-1 upf1*::*URA3*) complemented with either pRS315 or pRS315-UPF1.

Plasmid p2μm-*EST1* (pVL157; provided by V. Lundblad) contains a 2.58-kb *Bam*HI/*Sph*I fragment of *EST1* inserted in the *Bam*HI/*Sph*I site of YEp24. p2μm-*EST2* contains a 3.75-kb *Bam*HI-*Sac*I fragment containing the entire coding sequence of *EST2* in YEplac181 (15). p2μm-*EST3* contains a 1.15-kb fragment containing the wild-type *EST3* gene in YEplac181. p2μm-*STN1a* contains full-length *STN1* cloned as a *Pvu*II-*Sac*I fragment into the *Sma*I-*Sac*I sites in pRS423 (7). p2μm-*STN1b* contains full-length *STN1* cloned into YEplac195 (provided by M. Charbonneau). p2μm-*SAS2* (pDR1058; provided by D. Rivier) contains full-length *SAS2* cloned into pRS425. p2μm-*ORC5* (pAD002; provided by A. Dillon and J. Rine) contains *ORC5* cloned as a *Xho*I-*Not*I fragment into pFAT1. p2μm-*TEN1* (provided by M. Charbonneau) contains YLR010c and ~300 nucleotides (nt) of the promoter sequence. Strains containing two plasmids were made for all pairwise combinations of the seven relevant genes (*STN1*, *EST1*, *EST2*, *EST3*, *ORC5*, *SAS2*, and *TEN1*), except for *EST3* with *EST2*, *ORC5*, or *SAS2* and *ORC5* with *SAS2* or *TEN1*.

Strains expressing green fluorescent protein (GFP) reporter fusion mRNAs

were constructed by PCR-mediated homologous recombination (49). Each ORF was replaced from the start codon to the stop codon with the complete GFP ORF. P_{EST1}-GFP, P_{EST2}-GFP, and P_{EST3}-GFP were made by using pVL368, pVL296, and pVL298 (all provided by V. Lundblad), respectively, as templates. P_{STN1}-GFP was made by using pSE1393, which contains the *STN1* gene in YEplac181.

Telomere length and yeast telomerase assays. Average telomere length was determined by Southern blot analysis of *Pst*I-digested genomic DNA as described previously (25). To assess telomerase activity, yeast whole-cell extracts and DEAE fractions were prepared as previously described (8, 32). DEAE fractions were tested for telomerase activity by using two different primers in standard primer extension assays, and the results were quantified by PhosphorImager analysis (32).

RNA methods. For half-life and steady-state experiments, total RNA was extracted as described by Leeds et al. (21). To measure mRNA half-lives, strain AAY333 (provided by A. Atkin, University of Nebraska, Lincoln), which carries the *rbp1-1* temperature-sensitive allele encoding RNA polymerase II (36), was used. Transcription was terminated by shifting cells from 25 to 37°C. Cells were collected at intervals following temperature shift. After extraction, RNA was denatured by using glyoxal and dimethyl sulfoxide, separated on 1% agarose gels, and transferred to GeneScreen Plus (Dupont, NEN Research Products, Boston, Mass.) (4). RNA was detected by hybridization to radiolabeled DNA probes or riboprobes, which were prepared by in vitro transcription of template DNA in the presence of [α-^{32}P]UTP (800 or 3,000 Ci/mmol; Amersham Life Science, Arlington Heights, Ill.) by using the Riboprobe System (Promega Corp., Madison, Wis.). DNA templates for riboprobe synthesis were prepared by PCR amplification of genomic DNA by using standard conditions. The *ACT1* riboprobe is 224 nt in length and contains sequences complementary to nt 30 to 528 of the ORF. The *CDC13* riboprobe is 270 nt in length and contains sequences complementary to nt 2 to 271 of the ORF. The *CPA1* riboprobe is 697 nt in length and contains sequences complementary to nt 126 to 822 of the ORF. The *EST1* riboprobe is 691 nt in length and contains sequences complementary to nt 706 to 139 of the ORF. The *EST2* riboprobe is 645 nt in length and contains sequences complementary to nt 1011 to 1655 of the ORF. The *EST3* riboprobe is 597 nt in length and contains sequences complementary to nt −29 through 568 relative to the first nucleotide of the ORF. The GFP riboprobe is 363 nt in length and contains sequences complementary to nt 222 to 584 of the ORF. The *ORC5* riboprobe is 430 nt in length and contains sequences complementary to nt 506 to 935 of the ORF. The *PGK1* riboprobe is 95 nt in length and contains sequences complementary to nt 1157 to 1251 of the ORF. The *SAS2* riboprobe is 308 nt in length and contains sequences complementary to nt 353 to 660 of the ORF. The *STN1* riboprobe is 377 nt in length and contains sequences complementary to nt 551 to 927 of the ORF. The *TEN1* riboprobe is 366 nt in length and contains sequences complementary to nt 2 to 344 of the ORF. By using template DNA fragments obtained from restriction digestion or PCR, *CYH2* or *TRP1* probes were prepared and hybridized as described previously (2).

For analysis of *TLC1* RNA levels in telomerase fractions, the *TLC1* gene (nt 1 to 1301) was amplified by PCR and cloned between the *Bam*HI and *Eco*RV sites of pBluescript II KS+. The resulting plasmid was linearized by digestion with *Hin*fI, and antisense RNA encompassing residues 1097 to 1301 of the *TLC1* gene was generated by T3 RNA polymerase in the presence of 12 μM [α-^{32}P] GTP (31). Total RNAs from DEAE fractions were isolated and combined with the probe (100,000 cpm), precipitated with ethanol, hybridized, digested with RNase T_1, RNase A, and proteinase K, and analyzed by gel electrophoresis (33).

RESULTS AND DISCUSSION

Steady-state levels of *EST1*, *EST2*, *EST3*, *STN1*, *TEN1*, *SAS2*, and *ORC5* mRNA are elevated in *upf* mutants. We hypothesized that NMD regulates the steady-state mRNA levels of specific wild-type genes that are important for telomere function (25). To identify wild-type mRNAs that accumulate in *upf* mutant strains and are responsible for the associated telomere-related phenotypes, we focused on HDOA data for a subset of ~80 ORFs that encode proteins with known or suspected telomere function (Table 1) (23). Those with a combined knockout index (CKI) score of >0.5 or <−0.5 were selected for analysis. The CKI score is an indicator of how consistently the level of a specific mRNA is elevated or decreased in *upf* mutant strains relative to that in an isogenic *UPF* parental strain (23). By this criterion, the mRNA levels of the majority of the selected set of telomere-related mRNAs were not significantly affected in *upf* mutants. However, 7 of the ~80 mRNAs (encoded by *EST1*, *EST2*, *STN1*, *TEN1*, *SAS2*, *ORC5*, and *MSI1*/*CAC3*) had CKI scores of >0.5, with the average change in mRNA levels in *upf* mutants being an increase of 1.75- to 3.3-fold relative to the levels in *UPF* strains (Table 1). None of the mRNAs had a score of <−0.5. To confirm the results of the HDOA studies and to extend them to relevant RNAs not present on the HDOA, we compared steady-state levels of mRNAs in total RNA prepared from wild-type and *upf* mutant strains on Northern blots. As predicted from the HDOA experiments, the levels of *EST1*, *EST2*, *STN1*, and *TEN1* mRNA in the *upf* strains were increased by at least twofold relative to those in wild-type strains (Table 2).

TABLE 2. Average increases (*n*-fold) in mRNA levels measured by HDOA and Northern blot analysis

Gene name	HDOA data[a]	Northern blotting (*upf1 upf2 upf3*/*UPF1 UPF2 UPF3*)[b]	Northern blotting (*upf1*/*UPF1*)[c]	GFP reporter Northern blotting (*upf3*/*UPF3*)[d]
EST1	2.1 ± 0.4	2.8 ± 0.4	3.6	2.1 ± 0.61
EST2	2.3 ± 1.1	4.3 ± 0.4	7.7	1.8 ± 0.01
EST3	NT	3.1 ± 0.3	4.7	2.0 ± 0.01
ORC5	1.7 ± 0.3	2.0 ± 0.3	2.6	ND
SAS2	3.2 ± 0.9	2.9 ± 0.4	2.8	ND
STN1	2.6 ± 0.5	5.2 ± 0.5	6.1	3.1 ± 0.1
TEN1	3.3 ± 0.7	4.8 ± 0.7[e]	ND[f]	ND
CDC13	1.1 ± 0.7	1.1 ± 0.2	1.1	ND
MSI1	2.1 ± 1.0	1.3 ± 0.3	1.4	ND
TLC1	NT	0.8 ± 0.3[e]	0.9	ND

[a] Values represent average fold change and sample standard deviation (Table 1). NT, *EST3* and *TLC1* were not represented on the microarrays.
[b] mRNA levels were determined by using quantitative RNA blots like those shown in Fig. 1 for isogenic *upf* mutant strain LRSy307 (pRS316) compared with wild-type strain LRSy307 (pML1). Values represent average of data ± standard deviation from the four *upf* mutant strains relative to the wild type ($n = 4$). In all cases, mRNA levels were normalized to actin mRNA levels.
[c] mRNA levels were determined by using quantitative RNA blots of strains ML51 (*upf1* mutant) and ML34 (*UPF1*$^+$).
[d] GFP reporter mRNA levels were determined by quantitative RNA blots of strains YJB3758 and YJB4468.
[e] *TEN1* and *TLC1* RNA levels were determined in strain YJB1471 (*upf2* mutant) and were normalized to levels of *PGK1* mRNA.
[f] ND, not done.

EST1 and *EST2* (24) are members of the telomerase epistasis group. *EST2* encodes the catalytic subunit of telomerase. Est1p mediates the access of telomerase to the telomere through interactions with Cdc13/Est4p (11, 17, 43) and is required for in vivo, but not in vitro, telomerase activity (26). *CDC13*/*EST4* mRNA levels in *upf* mutant strains were unchanged (Table 2). *EST3* and *TLC1*, also members of the telomerase/*EST* epistasis group, were not present on the HDOA.

TLC1 encodes the RNA component of telomerase and was not included on the HDOA because it does not have an obvious ORF (47). High levels of *TLC1* RNA, like *upf* mutations, cause telomere shortening and reduced telomeric silencing (47). Northern analysis of total RNA indicated that *TLC1* RNA levels were not affected by *upf* mutations (Table 2), which is consistent with the idea that the NMD pathway acts on mRNAs during translation (2, 3) and not on untranslated RNAs like the product of *TLC1*. Thus, despite the similarity in the phenotypes of *upf* mutants and strains expressing high

levels of *TLC1* RNA, NMD does not affect telomere function by altering the steady-state levels of *TLC1* RNA.

The *EST3* ORF includes a programmed +1 frameshift (34) but was not included on the HDOA. Because *EST3* includes a frameshift within the 5′ 50% of the mRNA that bypasses a premature stop codon, it was a good candidate for an mRNA that is degraded by the NMD pathway (21, 22). The result of Northern blot analysis was consistent with this prediction: *EST3* mRNA levels in *upf* mutants increased approximately three- to fivefold relative to those in the isogenic *UPF* strains (Table 2).

STN1 and *TEN1* are essential genes and have a role in chromosome capping and the prevention of deleterious degradation of chromosome ends (18, 19). *STN1* encodes a high-copy suppressor of *cdc13-1* (19), and *TEN1* encodes a gene product that interacts physically with both Cdc13p and Stn1p to enhance the ability of Stn1p to negatively regulate telomerase activity at telomeres (18). Northern blot analysis confirmed that the mRNA levels of *STN1* and *TEN1* were elevated in *upf* mutant strains (Table 2). Thus, the mRNA levels of several telomerase subunits and regulators (Est1p, Est2p, Est3p, Stn1p, and Ten1p) are influenced by the NMD pathway, while the RNAs for others involved in the same telomere-related processes (*TLC1* RNA and Cdc13p) are not.

In addition to ORFs with known effects on telomerase function, data from the HDOA analysis revealed three genes (*SAS2*, *ORC5*, and *MSI1*/*CAC3*) with known effects on telomeric silencing and chromatin structure that had elevated mRNA levels in *upf* mutant strains. The results from Northern blot experiments were consistent with an increase in *SAS2* and *ORC5* mRNA levels of at least twofold (Table 2). Sas2p is a putative histone acetyltransferase that is a positive regulator of silencing at telomeres (44). *ORC5* encodes a component of the origin recognition complex (ORC) (10, 28) and influences silencing at telomeres and the HM loci (14). *MSI1*/*CAC3* mRNA, which encodes a subunit of chromatin assembly factor I, exhibited only a 1.3-fold increase on the Northern blots compared with the 2.1 ± 1.0-fold increase measured with HDOAs (Table 2). Therefore, we did not study *MSI1*/*CAC3* further. Also consistent with the HDOA data, the levels of *RAP1*, *SIR3*, *SIR4*, histone H4 (*HHF1* and *HHF2*), and *CAC1*/*RLF2* mRNAs in *upf* mutant strains did not change (data not shown). Thus, the NMD pathway affects the accumulation of mRNAs for at least seven genes (*EST1*, *EST2*, *EST3*, *STN1*, *TEN1*, *SAS2*, and *ORC5*) that are important for telomerase activity, telomere length control, and/or telomeric silencing.

Elevated levels of Est2p and Stn1p together phenocopy the telomeric silencing defect of *upf* mutant strains. To test the hypothesis that the telomere-related phenotypes of *upf* mutants are caused by increases in the level(s) of one or more of the seven mRNAs (*EST1*, *EST2*, *EST3*, *STN1*, *TEN1*, *SAS2*, and *ORC5*), we investigated whether increasing the copy number of any one of the genes could mimic (phenocopy) the telomere-related phenotypes of *upf* mutants. Yeast 2μm vectors were used to provide multiple copies of each of the individual genes with their native promoters. Northern analysis confirmed that the levels of the individual mRNAs increased twofold or more in strains carrying these 2μm plasmids (data not shown). Thus, the increased steady-state level of mRNA in these experiments generally equaled or exceeded the magnitude of the increase of specific mRNAs seen in *upf* mutants. Using strains that overexpress the gene products, we first examined whether increased copies of any individual gene could reduce the normal silencing of a telomere-adjacent gene. In wild-type strains, a *URA3* gene inserted near the left end of chromosome VII is subject to epigenetic telomeric silencing such that a proportion of the cells are able to grow on 5-FOA (synthetic dextrose complete [SDC] plus 5-FOA), which is toxic to Ura^+ strains. In *upf* mutant strains, the telomere-adjacent *URA3* is no longer silent; thus, *upf* mutant cells do not grow on SDC plus 5-FOA (Fig. 1). An elevated level of *EST2*, *EST3*, *STN1*, *SAS2*, or *ORC5* expression had no obvious effect on silencing of the telomeric *URA3* gene in these otherwise wild-type strains (Fig. 1, rows a to h). Similar results were observed when extra copies of *TEN1* or *EST1* were provided and telomeric silencing was monitored by use of a strain in which *ADE2* was inserted near the right end of chromosome V (data not shown). This is consistent with the observation that the overproduction of wild-type Est1p or Est2p does not affect telomeric silencing (13). Thus, an elevated level of any one of these mRNAs was not sufficient to account for the telomeric silencing phenotype of *upf* mutant strains.

We next tested whether overexpression of pairs of genes could confer a reduced silencing phenotype (similar to the

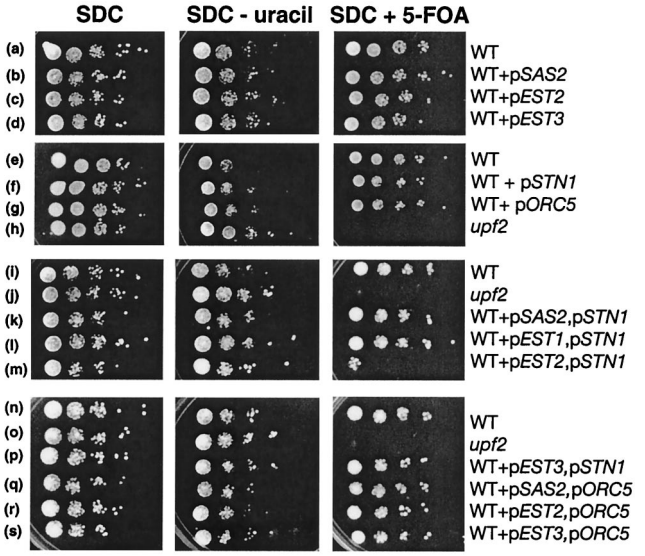

FIG. 1. High-level expression of *EST2* and *STN1* results in reduced silencing of telomere-adjacent genes. The expression of a telomere-adjacent *URA3* gene present in otherwise wild-type or *upf* mutant strains was determined by growth of the strain on complete medium (SDC), on medium lacking uracil (SDC − uracil), and on complete medium containing 5-FOA (SDC + 5-FOA) as indicated. Wild-type strain YJB487 was transformed with vector YEplac181 (row a), p2μm-*SAS2* (row b), p2μm-*EST2* (row c), p2μm-*EST3* (row d), vector pRS423 (row e), p2μm-*STN1* (row f), or p2μm-*ORC5* (row g). The same wild-type strain was cotransformed with both YEPlac181 and pRS423 (rows i and n) or p2μm-*SAS2* and p2μm-*STN1* (row k), p2μm-*EST1* and p2μm-*STN1* (row l), p2μm-*EST2* and p2μm-*STN1* (row m), p2μm-*EST3* and p2μm-*STN1* (row p), p2μm-*SAS2* and p2μm-*ORC5* (row q), p2μm-*EST2* and p2μm-*ORC5* (row r), or p2μm-*EST3* and p2μm-*ORC5* (row s). *upf2* mutant strain YJB539 was also transformed with YEplac181 (row h) or with both YEplac181 and pRS423 (rows j and o).

telomeric silencing phenotype of *upf* mutants). In each case, there was a greater-than-twofold average increase in the level of each RNA expressed (data not shown). Interestingly, only one combination of two plasmids, p2μm-*STN1* with p2μm-*EST2*, resulted in reduced telomeric silencing (Fig. 1, rows i to s). In several independent transformants, telomeric silencing in strains containing both p2μm-*STN1* and p2μm-*EST2* (Fig. 1, row m) was similar to that seen in *upf* mutants, as indicated by an inability to grow in the presence of 5-FOA. Because the NMD pathway down-regulates *PPR1* (21, 38), which is a positive regulator of *URA3*, the slight difference in growth observed for these two strains is most likely due to increased levels of *PPR1* and *URA3* expression in the *upf* mutant strains relative to their levels in the *UPF* strain containing both p2μm-*STN1* and p2μm-*EST2*. However, the effect of extra copies of p2μm-*STN1* and p2μm-*EST2* was seen when telomeric silencing was detected using *ADE2* to mark the right end of chromosome V. Thus, the effect is not dependent on *URA3* or *PPR1*. We did not observe additional silencing when p2μm-*TEN1* was expressed together with p2μm-*EST2* or p2μm-*STN1* by using the telomeric *ADE2* marker on chromosome V (data not shown). Thus, extra copies of *EST2* and *STN1* together, which encode the catalytic subunit of telomerase and a regulator of telomerase function, respectively, were sufficient to recapitulate the telomeric silencing defect observed in *upf* mutant strains.

Our results support a connection between telomerase regulation and telomeric silencing and are consistent with the idea that titration of telomere-associated proteins influences telomeric silencing. *TLC1* was cloned as a high-copy disruptor of telomeric silencing (47). This occurs through a 48-nt stem-loop structure in *TLC1* RNA that most likely interacts with the Ku proteins (40), which are important for telomere organization within the nucleus as well as for telomeric silencing (20). Similarly, in *upf* mutant strains, high levels of Stn1p and Est2p may perturb telomeric silencing by altering the stoichiometry or function of telomere-associated proteins such as Cdc13p and/or Ku (17).

Increased levels of Stn1p phenocopy the telomeric length control defect of *upf* mutant strains. We next investigated whether additional copies of *EST1*, *EST2*, *EST3*, *STN1*, *TEN1*, *SAS2*, or *ORC5* affected telomere length control to the degree seen in *upf* mutants. Telomere length was measured by digestion of genomic DNA with *Pst*I, which releases a terminal ~0.8-kb telomere fragment from the majority of the telomeres (those that contain a Y′ telomere-associated sequence [30]). Larger fragments that hybridize to the $TG_{1-3}/C_{1-3}A$ probe correspond to non-Y′ telomeres and to subtelomeric fragments that include internal $TG_{1-3}/C_{1-3}A$ repeats. The *upf* mutant strains carrying one or two control vectors exhibited an average telomere length that was ~57 bp shorter than that in an isogenic wild-type strain (Fig. 2) (Table 3). Transformation with p2μm-*STN1* alone resulted in a consistent decrease in the lengths of the shortest terminal telomere fragments (~43 ± 19 bp shorter than those in the wild type [Fig. 2] [Table 3]), which were significantly different from the lengths of telomeres in the wild-type strain but not significantly different from the lengths of telomeres in *upf* mutant strains. In contrast, increasing the level of *EST1*, *EST2*, *EST3*, *SAS2*, *ORC5*, or *TEN1* had no significant effect on telomere length in an otherwise wild-type strain (Fig. 2 and data not shown). Importantly, telomeres in strains that carried p2μm-*STN1* together with either p2μm-*TEN1* or p2μm-*EST2* were 54 ± 26 bp and 52 ± 20 bp shorter, respectively, than wild-type telomeres (Fig. 2) (Table 3). When p2μm-*STN1*, p2μm-*EST2*, and p2μm-*TEN1* were all present in the same strain, telomeres were 67 ± 28 bp shorter than in the wild-type and 10 ± 28 bp shorter than in the *upf* mutant strains. Since the levels of all three RNAs in this strain are higher than their levels in the *upf2* mutant strain, this result suggests that increased levels of *STN1* are required for the short-telomere phenotype of *upf* mutant strains and that increased levels of *TEN1* and *EST2* mRNAs contribute to the phenotype. Consistent with this notion, both Stn2p and Ten1p are negative regulators of telomerase (17, 18).

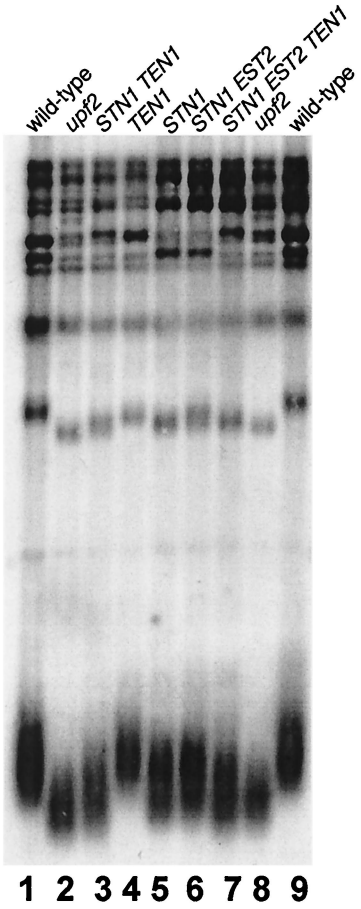

FIG. 2. High-level expression of *STN1* and *TEN1* results in reduced telomere length. Genomic DNA was digested with *Pst*I and analyzed on a 1% agarose gel, and telomere sequences were detected with a telomere repeat sequence probe on pCA75 (51). Lanes 1 and 9, wild-type strain (YJB209) transformed with control vector (YEp195) and crossed to YJB3011; lanes 2 and 8, *upf2* mutant strain (YJB7178); lanes 3, 4, and 7, YJB209 transformed with p2μm-*TEN1* (YLR010) and crossed with strains YJB3234, YJB3011, and YJB3345, respectively; lanes 5 and 6, YJB209 transformed with YEplac195 and crossed with YJB3234 and YJB3345, respectively.

Three NMD-sensitive genes, *STN1*, *TEN1*, and *EST2*, appear to account for the telomeric phenotypes of *upf* mutant strains. The effect of NMD on telomere length requires *STN1*, and the effect of NMD on telomeric silencing involves *STN1*

TABLE 3. Average telomere length in strains carrying extra copies of *STN1*, *TEN1*, and *EST2*

Relevant genotype[a]	Avg length[b]	Difference (bp) from:	
		Wild type	*upf2* mutant
WT	703 ± 21 (14)	0	57
upf2	646 ± 9 (6)	−57	0
STN1	660 ± 19 (6)[c]	−43	14
TEN1	723 ± 23 (6)[d]	20	77
STN1 TEN1	649 ± 26 (12)[c]	−54	3
STN1 EST2	651 ± 20 (6)[c]	−52	5
STN1 EST2 TEN1	636 ± 28 (6)[c]	−67	−10

[a] Genotypes as described in the legend to Fig. 2. Genes shown in capital letters were provided on 2μm plasmids.
[b] Average length in base pairs of *PstI* fragment ± standard deviation. The numbers in parentheses indicate the numbers of independent experiments done.
[c] Not significantly different from the *upf2* mutant according to the rank sum test (47).
[d] Not significantly different from wild type according to the rank sum test (47).

and *EST2*. Thus, NMD confers the two phenotypes through increased levels of *STN1* RNA in combination with other gene products. The involvement of Stn1p in both telomeric silencing and telomeric length control is especially interesting in light of the proposed role for Stn1p as the primary effector of chromosome end protection (39). Through interactions with Cdc13p, Stn1p also has a role in coupling lagging-strand synthesis (of the telomeric C strand) to telomerase extension of the 3′ end (telomeric G strand) of the chromosome (6). Thus, NMD-mediated control of the *STN1* mRNA level appears to be critical for both the telomere length and telomeric silencing phenotypes of *upf* mutant strains.

Sequences necessary for NMD-mediated control of telomere-related mRNA abundance. The NMD pathway controls the levels of specific mRNAs either directly or indirectly. Direct effects, in which the decay rate of an mRNA is affected by NMD, result when an mRNA contains a built-in premature stop codon. In wild-type mRNAs that are normally subject to NMD, this may occur when a translatable upstream ORF is present in the 5′ leader (46) or when leaky scanning leads to translation initiation at an out-of-frame AUG codon which brings a premature stop codon into register (50). Indirect effects on mRNA accumulation can result when the mRNA is transcriptionally regulated by the product of another mRNA that is affected by the NMD pathway (9, 23). Thus, mRNAs involved in telomere function could be either direct targets whose mRNA decay rates depend on NMD or indirect targets whose transcription rates depend on NMD.

We determined that NMD affects the expression of seven genes important for telomere functions and that a subset of these genes (*EST2*, *STN1*, and *TEN1*) can phenocopy a *upf* mutant strain. Because *STN1* contributed significantly to both of the telomere-related phenotypes of *upf* mutant strains, experiments were done to address the effect of NMD on the *STN1* mRNA levels. To determine whether *STN1* mRNA is specifically targeted for degradation by NMD, we compared the half-lives of *STN1* mRNA in wild-type and *upf1* mutant strains. The half-lives of *STN1* mRNA were not significantly different when measured in *UPF1* and *upf1* strains (Fig. 3A), whereas the control *CPA1* mRNA (50) exhibited an approximately fourfold difference (Fig. 3C), indicating that *STN1* mRNA does not appear to be a direct substrate of NMD. If *STN1* mRNA is indirectly regulated by one or more NMD-sensitive transcription factors, then the *STN1* promoter sequence is expected to confer NMD control to a reporter gene inserted in place of the *STN1* coding sequence. To determine whether the *STN1* promoter is sufficient for NMD control of the mRNA, we constructed a plasmid that fused 300 nt of DNA upstream to and including the *STN1* start codon to the ORF for GFP (P_{STN1}-GFP) (27). Epifluorescence microscopy indicated that wild-type cells carrying P_{STN1}-GFP appeared slightly green due to expression of GFP from the *STN1* sequence (data not shown). These cells were crossed to a *upf3*::*HIS3* strain and sporulated. Epifluorescence analysis of asci containing four spores revealed an apparent segregation of two bright green and two dim green spores (data not shown). Northern blot analysis of RNA levels in sister spores from this cross (using a GFP riboprobe) indicated that levels of reporter

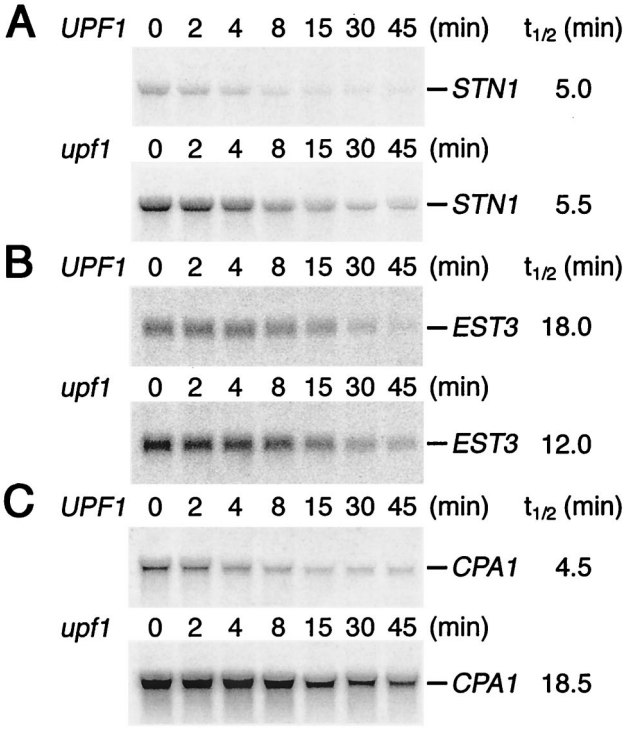

FIG. 3. *STN1* and *EST3* mRNA half-lives in wild-type and *upf1* mutant strains are not different. mRNA half-lives ($t_{1/2}$) were determined by Northern blot analysis of total RNA (15 μg) extracted from *UPF1* (AAY333 plus pRS315UPF1) and *upf1* mutant (AAY333 plus vector) strain cultures collected at the indicated times after termination of transcription (see Materials and Methods). Blots were hybridized with a riboprobe complementary to the *STN1* mRNA (A), *EST3* mRNA (B), or *CPA1* mRNA (C). In two independent experiments, the *STN1* mRNA at time zero was 3.4- and 4.0-fold more abundant in the *upf1* mutant strain. Similarly, the *EST3* mRNA was 2.0- and 2.1-fold more abundant, and the *CPA1* mRNA was 4.1- and 3.8-fold more abundant in the *upf1* mutant strain. Half-life values determined from the experiment shown are indicated at right. In independent experiments, the *STN1* half-lives were 5.0 min in the *UPF1* strain and 4.5 min in the *upf1* mutant strain, the *EST3* half-lives were 12.0 min in the *UPF1* strain and 15.0 min in the *upf1* mutant strain, and the *CPA1* half-lives were 4 min in the *UPF1* strain and 16 min in the *upf1* mutant strain.

mRNA were 3.1-fold higher in *upf3* mutant strains than in *UPF3* sister spores (Table 2). Analysis of isogenic *upf3*::*HIS3* and *UPF3* progeny by fluorimetry confirmed that *upf3*::*HIS3* spores emitted significantly more GFP fluorescence than the *UPF3* spores (data not shown). These results indicate that the *STN1* promoter sequences are sufficient to confer NMD-dependent control on *STN1* mRNA levels. Taken together, our results suggest that the promoter of *STN1* is subject to control by NMD through an indirect mechanism involving the modulation of transcription levels. We propose that NMD controls the stability of an mRNA corresponding to an upstream regulator of *STN1* expression.

Using GFP-reporter fusions, we compared *EST1* and *EST2* expression in wild-type and *upf* mutant strains. We constructed and analyzed the expression of a fusion construct containing *EST1* sequence 5′ to the start codon fused to the GFP ORF. GFP fluorescence levels were too low for quantitation by epifluorescence microscopy, fluorimetry, or flow cytometry. Northern analysis revealed a twofold increase in the levels of reporter mRNA in *upf3* spores relative to the levels in *UPF3* sister spores (Table 2), suggesting that, like *STN1*, promoter sequences 5′ to the *EST1* ORF contribute to the increased level of *EST1* mRNA in *upf* mutant strains. Similar results were obtained when the *EST2* ORF was replaced with the GFP ORF: the reporter mRNA levels were 1.8-fold higher in *upf3* mutants than in *UPF3* strains (Table 2). This indicates that sequences 5′ of the *EST1* and *EST2* ORFs are sufficient to account for all of the *EST1* and most of the *EST2* mRNA accumulation, respectively, in *upf* mutant strains relative to the levels in wild-type strains. However, we could not discern whether *EST1* and *EST2* mRNAs are regulated directly or indirectly, because the mRNA half-lives could not be measured.

The *EST3* ORF was also replaced with that of GFP, and the accumulation of reporter mRNA levels was twofold greater in *upf3* mutants than in *UPF3* strains (Table 2), indicating that the increased level of *EST3* mRNA in *upf* mutant strains was not due to sequences within the *EST3* ORF. This was surprising because the *EST3* ORF has an internal stop codon and a +1 programmed frameshift (34), suggesting that it might be degraded by NMD. Furthermore, the *EST3* mRNA decay rates in *upf1* mutant and *UPF1* strains were similar (Fig. 3B), indicating that *EST3* is not regulated at the level of mRNA decay. This implies that the internal stop codon in the ORF is not responsible for the effects of NMD on this mRNA. This in-frame stop codon could fail to trigger NMD for several reasons, including its position within the ORF relative to downstream sequences required for NMD or an interplay between the internal stop codon and the programmed frameshift site that allows translation to bypass the stop codon frequently. In either case, *EST3* appears to be affected by NMD because of indirect effects on *EST3* transcription initiation.

Role of NMD in controlling the level of telomerase activity. To determine whether telomere length in *upf* mutant strains was due to altered telomerase activity, we compared the ability of partially purified telomerase extracts prepared from wild-type and *upf2* mutant strains to extend telomere sequence primers. Extracts from the two strains exhibited nearly identical activity in the primer extension assays with two different oligonucleotides as substrates (Fig. 4a). Consistent with the results of our Northern blot analysis of total RNA (Table 2),

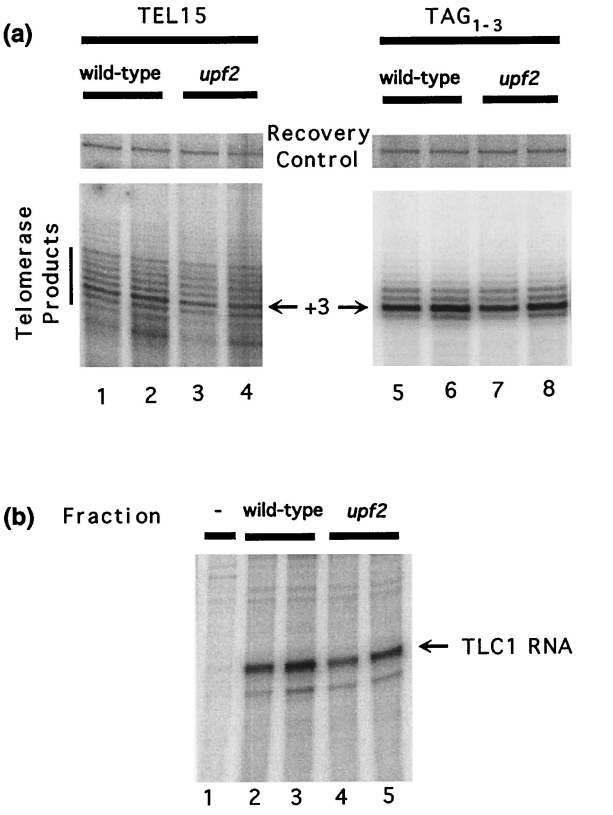

FIG. 4. In vitro telomerase activity and *TLC1* RNA levels are not dependent on *UPF2*. (a) Primer extension assays for yeast telomerase were carried out using either TEL15 primer (lanes 1 to 4) or TAG_{1-3} primer (lanes 5 to 8) and either l μl (lanes 1, 3, 5, and 7) or 10 μl (lanes 2, 4, 6, and 8) of DEAE fractions derived from wild-type (YJB209; lanes 1, 2, 5, and 6) or *upf2* mutant (YJB1274; lanes 3, 4, 7, 8) strains. Each reaction also contained a prelabeled 46-mer oligonucleotide that served as a recovery control. Under the standard reaction conditions, telomerase consistently gave rise to the product at the primer +3 position (arrows). (b) RNase protection assays using either 100 μl (lanes 2 and 4) or 200 μl (lanes 3 and 5) of DEAE fractions derived from either wild-type (YJB209; lanes 2 and 3) or *upf2* mutant (YJB1274; lanes 4 and 5) strains. Lane 1 contains 20 μg of yeast tRNA as a negative control (48).

the amounts of *TLC1* RNA in partially purified telomerase extracts were comparable in the two strains (Fig. 4B). Thus, loss of NMD (and the resulting increased levels of *EST2* mRNA) does not affect the amount of extractable telomerase activity or the amount of *TLC1* RNA in the telomerase fraction. In *S. cerevisiae*, in vitro telomerase activity requires Est2p and *TLC1* but does not require Est1p or Est3p (26). Since neither in vitro telomerase activity nor the amount of *TLC1* RNA is altered in *upf* mutant strains, it is possible that extractable telomerase activity in *upf* mutant cells may be limited by the levels of *TLC1* RNA.

In vivo, *EST1*, *EST3*, *STN1*, and *TEN1* contribute to telomerase-dependent telomere length control, presumably by regulating the access of the chromosomal terminus to telomerase (6, 17, 18, 39; reviewed in reference 12). Despite the fact that the NMD pathway controls levels of *EST2* mRNA, which encodes the catalytic subunit of telomerase, telomere length and levels of extractable telomerase activity are not affected by

increased levels of *EST2* mRNA (Fig. 2 and 3). Thus, the shorter telomeres in *upf* mutant strains are not due to increased levels of *EST2* or to increased levels of telomerase activity. Rather, we propose that *upf* mutant strains have short telomeres because Stn1p (together with Ten1p) limits the accessibility of the telomeres to telomerase. Our results suggest that NMD affects telomeric silencing by increasing the levels of Stn1p and Est2p, which may titrate other factors that interact with the chromosome end complex and/or with telomerase itself. One candidate for the titrated factor is Cdc13p, which interacts with both telomerase and Stn1p (17, 39). Another candidate is the Ku70/Ku80 complex, which is also required for telomere length control and telomeric silencing (5, 20, 35, 41).

In summary, the level of expression of several telomerase components and regulators, including *EST1*, *EST2*, *EST3*, *STN1*, and *TEN1*, but not *CDC13*/*EST4*, depend on NMD. Increasing the levels of *EST2*, *STN1*, and *TEN1*, which encode the catalytic subunit of telomerase and two negative regulators of telomerase recruitment to the telomere, is sufficient to account for the telomeric silencing and telomere shortening phenotypes of *upf* mutants. While levels of mRNAs that regulate telomerase are altered in *upf* mutants, the levels of *TLC1* RNA and of in vitro telomerase activity are not changed. This implies that the telomere length phenotype of *upf* mutants is due to changes in the access of telomerase to the telomere rather than to changes in the amount of telomerase activity. For *EST1*, *EST2*, *EST3*, and *STN1*, the sequences upstream of the ORF are sufficient to confer an NMD-mediated effect upon reporter mRNA levels. The effect of NMD on *STN1* and *EST3* mRNA levels most likely occurs via transcription initiation, since mRNAs were not stabilized in *upf* mutant strains. For *EST3*, this implies that the +1 programmed frameshift in the Est3p coding sequence (34) does not trigger *UPF*-mediated mRNA decay. We propose that NMD controls the mRNA stability for one or more upstream regulators of *STN1* and possibly *EST3*. Thus, the NMD pathway affects telomere length and telomeric silencing by regulating the levels of *EST2*, *STN1*, and *TEN1* mRNAs, primarily through an indirect effect on transcription levels.

ACKNOWLEDGMENTS

J.N.D. and J.L.-S. contributed equally to this publication.

We thank Sara Johnson and Angela Williams for technical assistance; Audrey Atkin for strain AAY333; M. Charbonneau, A. Dillon, V. Lundblad, and J. Rine for providing plasmids, and Kirk Anders for helpful discussions regarding Ppr1p.

This work was supported by grants from the National Institutes of Health (GM38636 to J.B. and GM65172 to M.R.C.), the National Science Foundation (MCB-9870313 to M.R.C. and MCB-0091300 to J.N.D.), and the American Cancer Society (RPG-99-048-01-GMC to N.L.). A.F. was supported by the McIntyre-Stennis HATCH grant WIS04308 awarded to M.R.C.

REFERENCES

1. **Altamura, N., O. Groudinsky, G. Dujardin, and P. P. Slonimski.** 1992. *NAM7* nuclear gene encodes a novel member of a family of helicases with a Zn-ligand motif and is involved in mitochondrial functions in *Saccharomyces cerevisiae*. J. Mol. Biol. **224:**575–587.
2. **Atkin, A. L., N. Altamura, P. Leeds, and M. R. Culbertson.** 1995. The majority of yeast UPF1 co-localizes with polyribosomes in the cytoplasm. Mol. Biol. Cell **6:**611–625.
3. **Atkin, A. L., L. R. Schenkman, M. Eastham, J. N. Dahlseid, M. J. Lelivelt, and M. R. Culbertson.** 1997. Relationship between yeast polyribosomes and Upf proteins required for nonsense mRNA decay. J. Biol. Chem. **272:**22163–22172.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1993. Current protocols in molecular biology. Greene Publishing Associates and Wiley Interscience, New York, N.Y.
5. **Boulton, S. J., and S. P. Jackson.** 1998. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. EMBO J. **17:**1819–1828.
6. **Chandra, A., T. R. Hughes, C. I. Nugent, and V. Lundblad.** 2001. Cdc13 both positively and negatively regulates telomere replication. Genes Dev. **15:**404–414.
7. **Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero, and P. Hieter.** 1992. Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.
8. **Cohn, M., and E. H. Blackburn.** 1995. Telomerase in yeast. Science **269:** 396–400.
9. **Culbertson, M. R.** 1999. RNA surveillance. Unforeseen consequences for gene expression, inherited genetic disorders and cancer. Trends Genet. **15:** 74–80.
10. **Dillin, A., and J. Rine.** 1998. Roles for ORC in M phase and S phase. Science **279:**1733–1737.
11. **Evans, S. K., and V. Lundblad.** 1999. Est1 and Cdc13 as comediators of telomerase access. Science **286:**117–120.
12. **Evans, S. K., and V. Lundblad.** 2000. Positive and negative regulation of telomerase access to the telomere. J. Cell Sci. **113:**3357–3364.
13. **Evans, S. K., M. L. Sistrunk, C. I. Nugent, and V. Lundblad.** 1998. Telomerase, Ku, and telomeric silencing in *Saccharomyces cerevisiae*. Chromosoma **107:**352–358.
14. **Fox, C. A., A. E. Ehrenhofer-Murray, S. Loo, and J. Rine.** 1997. The origin recognition complex, *SIR1*, and the S phase requirement for silencing. Science **276:**1547–1551.
15. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
16. **Gottschling, D. E., O. M. Aparicio, B. L. Billington, and V. A. Zakian.** 1990. Position effect at *S. cerevisiae* telomeres: reversible repression of pol II transcription. Cell **63:**751–762.
17. **Grandin, N., C. Damon, and M. Charbonneau.** 2000. Cdc13 cooperates with the yeast Ku proteins and Stn1 to regulate telomerase recruitment. Mol. Cell. Biol. **20:**8397–8408.
18. **Grandin, N., C. Damon, and M. Charbonneau.** 2001. Ten1 functions in telomere end protection and length regulation in association with Stn1 and Cdc13. EMBO J. **20:**1173–1183.
19. **Grandin, N., S. I. Reed, and M. Charbonneau.** 1997. Stn1, a new *Saccharomyces cerevisiae* protein, is implicated in telomere size regulation in association with Cdc13. Genes Dev. **11:**512–527.
20. **Laroche, T., S. G. Martin, M. Gotta, H. C. Gorham, F. E. Pryde, E. J. Louis, and S. M. Gasser.** 1998. Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. Curr. Biol. **8:**653–656.
21. **Leeds, P., S. W. Peltz, A. Jacobson, and M. R. Culbertson.** 1991. The product of the yeast *UPF1* gene is required for rapid turnover of mRNAs containing a premature translational termination codon. Genes Dev. **5:**2303–2314.
22. **Leeds, P., J. M. Wood, B. S. Lee, and M. R. Culbertson.** 1992. Gene products that promote mRNA turnover in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**2165–2177.
23. **Lelivelt, M. J., and M. R. Culbertson.** 1999. Yeast Upf proteins required for RNA surveillance affect global expression of the yeast transcriptome. Mol. Cell. Biol. **19:**6710–6719.
24. **Lendvay, T. S., D. K. Morris, J. Sah, B. Balasubramanian, and V. Lundblad.** 1996. Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional EST genes. Genetics **144:**1399–1412.
25. **Lew, J. E., S. Enomoto, and J. Berman.** 1998. Telomere length regulation and telomeric chromatin require the nonsense-mediated mRNA decay pathway. Mol. Cell. Biol. **18:**6121–6130.
26. **Lingner, J., T. R. Cech, T. R. Hughes, and V. Lundblad.** 1997. Three Ever Shorter Telomere (*EST*) genes are dispensable for *in vitro* yeast telomerase activity. Proc. Natl. Acad. Sci. USA **94:**11190–11195.
27. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:**953–961.
28. **Loo, S., C. A. Fox, J. Rine, R. Kobayashi, B. Stillman, and S. Bell.** 1995. The origin recognition complex in silencing, cell-cycle progression, and DNA replication. Mol. Biol. Cell **6:**741–756.
29. **Losson, R., R. P. Fuchs, and F. Lacroute.** 1985. Yeast promoters URA1 and URA3. Examples of positive control. J. Mol. Biol. **185:**65–81.
30. **Louis, E. J., and J. E. Haber** 1992. The structure and evolution of subtelomeric Y′ repeats in *Saccharomyces cerevisiae*. Genetics **131:**559–574.
31. **Lue, N. F., and R. D. Kornberg.** 1987. Accurate initiation at RNA polymerase II promoters in extracts from *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **84:**8839–8843.
32. **Lue, N. F., and Y. Peng.** 1998. Negative regulation of yeast telomerase

activity through an interaction with an upstream region of the DNA primer. Nucleic Acids Res. **26:**1487–1494.
33. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12:**7035–7056.
34. **Morris, D. K., and V. Lundblad.** 1997. Programmed translational frameshifting in a gene required for yeast telomere replication. Curr. Biol. **7:**969–976.
35. **Nugent, C. I., G. Bosco, L. O. Ross, S. K. Evans, A. P. Salinger, J. K. Moore, J. E. Haber, and V. Lundblad.** 1998. Telomere maintenance is dependent on activities required for end repair of double-strand breaks. Curr. Biol. **8:**657–660.
36. **Parker, R., D. Herrick, S. Peltz, and A. Jacobson.** 1991. Measurement of mRNA decay rates in *Saccharomyces cerevisiae*. Methods Enzymol. **194:**415–423.
37. **Peltz, S. W., A. H. Brown, and A. Jacobson.** 1993. mRNA destabilization triggered by premature translational termination depends on at least three *cis*-acting sequence elements and one *trans*-acting factor. Genes Dev. **7:**1737–1754.
38. **Peltz, S. W., and A. Jacobson.** 1993. mRNA turnover in *Saccharomyces cerevisiae*, p. 291–327. *In* G. Brawerman and J. Belasco (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
39. **Pennock, E., K. Buckley, and V. Lundblad.** 2001. Cdc13 delivers separate complexes to the telomere for end protection and replication. Cell **104:**387–396.
40. **Peterson, S. E., A. E. Stellwagen, S. J. Diede, M. S. Singer, Z. W. Haimberger, C. O. Johnson, M. Tzoneva, and D. E. Gottschling.** 2001. The function of a stem-loop in telomerase RNA is linked to the DNA repair protein Ku. Nat. Genet. **27:**64–67.
41. **Porter, S. E., P. W. Greenwell, K. B. Ritchie, and T. D. Petes.** 1996. The DNA-binding protein Hdf1p (a putative Ku homolog) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*. Nucleic Acids Res. **24:**582–585.
42. **Pulak, R., and P. Anderson.** 1993. mRNA surveillance by the *Caenorhabditis elegans smg* genes. Genes Dev. **7:**1885–1897.
43. **Qi, H., and V. A. Zakian.** 2000. The *Saccharomyces* telomere-binding protein Cdc13p interacts with both the catalytic subunit of DNA polymerase alpha and the telomerase-associated Est1 protein. Genes Dev. **14:**1777–1788.
44. **Reifsnyder, C., J. Lowell, A. Clarke, and L. Pillus.** 1996. Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat. Genet. **14:**42–49.
45. **Roy, A., and R. Losson.** 1990. *cis*- and *trans*-acting regulatory elements of the yeast *URA3* promoter. Mol. Cell. Biol. **10:**5257–5270.
46. **Ruiz-Echevarria, M. J., and S. W. Peltz.** 2000. The RNA binding protein Pub1 modulates the stability of transcripts containing upstream open reading frames. Cell **101:**741–751.
47. **Singer, M. S., and D. E. Gottschling.** 1994. *TLC1*: template RNA component of *Saccharomyces cerevisiae* telomerase. Science **266:**404–409.
48. **Snedecor, G. W., and W. G. Cochran.** 1980. Statistical methods, 7th ed. Iowa State University Press, Ames.
49. **Wach, A.** 1996. PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in *S. cerevisiae*. Yeast **12:**259–265.
50. **Welch, E. M., and A. Jacobson.** 1999. An internal open reading frame triggers nonsense-mediated decay of the yeast SPT10 mRNA. EMBO J. **18:**6134–6145.
51. **Wellinger, R. J., A. J. Wolf, and V. A. Zakian.** 1993. Origin activation and formation of single-strand TG_{1-3} tails occur sequentially in late S phase on a yeast linear plasmid. Mol. Cell. Biol. **13:**4057–4065.