Supporting Information

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SI Materials and Methods

Media, Growth Conditions, and RNA Extraction. Yeast strains were grown at 30 °C in YPD medium [1% (wt/vol) yeast extract, 2% (wt/vol) peptone, and 2% (wt/vol) glucose] until optical density at 600 nm (OD_{600}) of 0.3 was reached, unless otherwise noted. For the 5′ RLM-RACE and Northern blot analysis shown in Fig. 4*C*, yRP2875 carrying pRP2407 or pRP2408 was grown at 30 °C in synthetic complete (SC) medium lacking uracil but containing 2% (wt/vol) galactose. For the 5′ RLM-RACE and Northern blot analysis shown in Fig. S2*G*, yRP2875 carrying pRP2407 was grown essentially in the same way except that the translation inhibitor cycloheximide was added at the final concentration of 100 μg/mL when OD_{600} of 0.3 was reached. Portions of the cultures were collected at 15, 30, 60, and 120 min after the addition of cycloheximide. For the 5′ RLM-RACE analysis shown in Fig. 5*B*, strains yRP2867 and yRP2868 were grown at 30 °C in SC medium lacking uracil but containing 2% (wt/vol) glucose. Cells were pelleted by centrifugation and rapidly frozen in liquid nitrogen. At least two biological replicates were performed for each experiment. Total RNA was extracted from frozen cell pellets by a hot phenol method. Polyadenylated RNA was selected using Oligo (dT) DynaBeads (Invitrogen).

Yeast Strain Construction. To generate yRP2857 and yRP2858, the *XRN1* gene was disrupted in yRP2856 (W303-1A) and yRP2601 (W303-1B) with the *kanMX6* cassette by a PCR-based method (1). yRP2860 (*dcp2*Δ *xrn1*Δ) was constructed from a cross between yRP2858 (*xrn1*Δ) and yRP2859 (*dcp2*Δ). The *DBR1* gene was disrupted with the *hphMX* cassette (2) in yRP2856 (W303-1A) and yRP2601 (W303-1B) to generate yRP2861 and yRP2862. yRP2861 (*dbr1*Δ *xrn1*Δ), yRP2862 (*dbr1*Δ *dcp2*Δ), and yRP2863 (*dbr1*Δ *dcp2*Δ *xrn1*Δ) were constructed from a cross between yRP2860 (*dcp2*Δ *xrn1*Δ) and yRP2862 (*dbr1*Δ). The *GLR1* gene was disrupted with the *HIS3MX* cassette in yRP2856 (W303-1A) to generate yRP2874. To generate yRP2875 (*glr1*Δ *xrn1*Δ), the *XRN1* gene was disrupted with the *kanMX* cassette in yRP2874. The *RAI1* gene was disrupted with the *hphMX* cassette in yRP2601 (W303-1B) to generate yRP2866. Strains yRP2867 and yRP2868 were constructed in multiple steps. First, a heterozygous diploid strain (yRP2873) resulted from a cross between yRP2860 (*dcp2*Δ *xrn1*Δ) and yRP2866 (*rai1*Δ) was transformed with plasmids pRP2403 and pRP2404, and the transformants were sporulated. Haploid progenies were recovered on SC medium lacking uracil and histidine but containing 2% (wt/vol) glucose. Subsequently, *dcp2*Δ*rai1*Δ*xrn1*Δ strains were selected based on the ability to grow in the presence of Geneticin G418 (Teknova) and Hygromycin B (Calbiochem). Finally, mating type was checked by PCR using oRP1614, oRP1615, and oRP1616 (3). To generate yRP2869, yRP2870, and yRP2871, the diploid strain yRP2873 was transformed with pRP1892 and then sporulated. Haploid progenies were recovered on SC medium lacking uracil and histidine but containing 2% (wt/vol) glucose. A haploid *dcp2*Δ *rai1*Δ strain carrying pRP1892 was selected and transformed with pRS200, pRP2405, and pRP2406. To generate yRP2876, the *DCS1* gene was disrupted with the *hphMX* cassette in yRP2601 (W303-1B). yRP2877 (*dcs1*Δ *dcp2*Δ *xrn1*Δ) was constructed from a cross between yRP2860 and yRP2876. Gene disruption was checked using appropriate oligonucleotides (Table S3).

Plasmid Construction. To generate pRP2403 and pRP2405, a DNA fragment containing 848-bp upstream region, ORF, and 439-bp downstream region of *RAI1* was PCR amplified from genome using primers oRP1619 and oRP1620. The resultant fragment was digested with restriction enzymes SacI and XhoI and cloned into corresponding sites in pRS416 and pRP200. pRP2404 and pRP2405 were constructed in multiple steps. First, a DNA fragment was amplified from genome using primers oRP1619 and oRP1618. A second PCR was performed using primers oRP1617 and oRP1620. The two PCR fragments were then mixed and fused by PCR amplification using primers oRP1619 and oRP1620. The resultant fragment was digested with restriction enzymes SacI and XhoI and cloned into corresponding sites in pRS416 and pRS200. Note that pRP2406 contains an unintended silent mutation at the 453rd nucleotide in *RAI1* ORF. To generate pRP2407 and pRP2408, DNA fragments were PCR-amplified from genome using a forward primer oRP1660 and reverse primers oRP1661 and oRP1662, and cloned into pSH47 that was linearized with HindIII and XhoI, by gap repair.

Library Preparation for Illumina Sequencing. Libraries were constructed essentially as described previously (4). Briefly, a 5′ RNA adaptor oRP1627 that includes a recognition site for the MmeI restriction enzyme was selectively ligated to 5′ P species in poly (A) RNA fraction using T4 RNA ligase (Ambion). The ligated RNA was reverse-transcribed using oligo(dT) linked to a 3′ adaptor sequence (oRP1628), PCR-amplified for 5 cycles using primers oRP1629 and oRP1630, and treated with MmeI, which cleaves 20 or 21 nt 3′ of the recognition site. The digestion products were gel-purified, ligated to a 3′ double-strand DNA adaptor (oRP1631 and oRP1632), and again gel-purified. The resultant material was PCR-amplified using primers oRP1633 and oRP1634, gel-purified, and sequenced on the Illumina Genome Analyzer II or HiSeq 2000 using the primer oRP1635.

Alignment of Sequences. From the raw sequences obtained by the Illumina Genome Analyzer II and HiSeq 2000, read sequences composed of 20- or 21-nt-long captured RNA sequences, followed by the 3′ Illumina adaptor sequence were selected using fastx_barcode_splitter.pl in FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/download.html). The adaptor sequences were removed from the barcode-matched reads using Perl. Identical sequences were collapsed and counted using fastx_collapser in FASTX-Toolkit to generate a file of distinct sequences and occurrence counts. The resultant 20- or 21-nt-long sequences were aligned to reference sequences using Bowtie 0.12.7 (http://bowtie-bio.sourceforge.net) (5). Bowtie options were set so that two mismatches were allowed and alignments were required to be unique. The reference was composed of nuclear and mitochondrial genome assemblies of *S. cerevisiae* S288C strain (released on February 3, 2011), A364A 2-μm plasmid sequence [National Center for Biotechnology Information (NCBI) accession number NC_001398], dsRNA virus genome (killer virus M1, virus L-A and L-BC, single-stranded viruses 20S and 23S; NCBI accession numbers NC_001782, NC_003745, NC_001641, NC_004051, and NC_004050, respectively), and the sequence of the *kanMX6* cassette (1), with which the *XRN1* gene was disrupted. Summary statistics of high-throughput sequencing and mapping are shown in Fig. S1*C*.

5′ P Tag Profiling. For individual libraries 5′ P tag profiles of 6,603 yeast mRNAs (Dataset S1) were built by plotting the abundance of each sequence read as a function of its position in mRNA using a set of Perl programs. In this process, we exclusively used sequence reads that were aligned to the reference with no mis-

match. ORF annotations were downloaded from Saccharomyces Genome Database (SGD) (http://downloads.yeastgenome.org/curation/chromosomal_feature/SGD_features.tab) on May 11, 2012. UTR lengths were taken from a previous RNAseq study (6). Where UTR annotation was not available, the ORF start/end was considered to be the transcript start/end. Reproducibility of two independent WT experiments and three independent *dcp2*Δ *xrn1*Δ experiments was evaluated by the Pearson correlation coefficient when comparing the raw number of reads obtained for each nucleotide in all mRNAs for each pair of replicates (Fig. S1*D*). Read counts were normalized to the total reads mapping to all mRNAs. Normalized libraries from biological replicates were combined and averaged for further analysis.

Identification of 5′ P Peak Cites. Screen for statistically significant 5′ P peak sites in mRNA was performed essentially as described previously (7). First, the read counts within each mRNA were fit to a negative binomial distribution, and *P* values were computed for all sites based on the negative binomial fit for the transcript, using a program written in R (http://www.r-project.org/). The resultant *P* values were adjusted with the Benjamini–Hochberg false discovery rate (FDR) (<0.01) estimator (8). All 2,738 sites in the WT sample and 153,593 sites in *dcp2*Δ *xrn1*Δ with *P* values below the cutoff (FDR < 0.01) are listed in Dataset S2. To categorize these peaks, annotations of introns and intron-derived snoRNAs were obtained from SGD (http://downloads.yeastgenome.org/curation/chromosomal_feature/SGD_features.tab) on May 11, 2012.

RT-PCR. Poly(A) RNA was treated with RQ RNase-free DNaseI (Promega) at 37 °C for 1 h. The reaction was extracted with phenol/chloroform/isoamyl alcohol (IAA) and then with chloroform and precipitated with ethanol. A total of 100 ng of DNase-treated poly(A) RNA was incubated with 100 units of SuperScript III Reverse Transcriptase (Invitrogen) in a 10-μL reaction at 55 °C for 1 h. One-fiftieth of the resultant cDNA was PCR-amplified with HotStarTaq DNA polymerases (Qiagen) for 35 cycles using gene-specific primers (Table S3).

5′ RLM-RACE. A total of 250 ng of poly(A) RNA was incubated with 20 pmol of 5′ RACE RNA adaptor oRP1522 and 10 units of T4 RNA ligase (Ambion) in a 10-μL reaction at 37 °C for 1 h. One-tenth of the reaction was incubated with 200 units of SuperScript III Reverse Transcriptase (Invitrogen) in a 20-μL reaction at 55 °C for 1 h. One-twentieth of the resultant cDNA was PCR-amplified with HotStarTaq DNA polymerases (Qiagen) for 35 cycles using a primer corresponding to the adaptor sequence (oRP1523) and a gene-specific reverse primer (Table S3). The PCR products were run on 1.5% agarose gel and visualized negatively after ethidium bromide staining. For analysis shown in Fig. 2*C*, poly(A) RNA was treated with RQ RNase-free DNaseI (Promega) before RNA ligation. As a standard, *ACT1* mRNA was detected using oRP1637 and oRP1638.

cRT-PCR. cRT-PCR was performed essentially as described previously (9, 10). A total of 100 ng of DNase-treated poly(A) RNA was incubated with 180 units of T4 RNA ligase (New England Biolabs) in a 400-μL reaction at 16 °C overnight. The reaction was extracted with phenol/chloroform/IAA and then with chloroform and precipitated with ethanol. One-twentieth of reconstituted ligation product was incubated with 2 pmol of gene-specific primer (Table S3) and 200 units of SuperScript III Reverse Transcriptase (Invitrogen) in a 20-μL reaction at 55 °C for 2 h. One-twentieth of the resultant cDNA was PCR-amplified with HotStarTaq DNA polymerases (Qiagen) for 35 cycles using a pair of gene-specific primers (Table S3). As a standard, *ACT1* mRNA was detected using oRP1637 and oRP1638.

Analysis of Intron-Containing Genes. The *dcp2*Δ *xrn1*Δ library data were analyzed for 240 intron–containing genes that were selected from the 6,603 mRNAs (Dataset S1) based on intron annotations downloaded from SGD (http://downloads.yeastgenome.org/curation/chromosomal_feature/SGD_features.tab) on May 11, 2012. We excluded: (*i*) genes with zero 5′ P tag abundance; (*ii*) genes that contain more than one intron; (*iii*) genes that contain snoRNA in intron; and (*iv*) *HAC1*, which undergoes an unconventional splicing. Abundance of 5′ P tag at 5′ SS and within a 5-nt-long region just downstream of the 5′ SS (A_{5ss}) was computed and divided by 6, the length of the region ($A_{5ss}/6$). As a measure of transcript level in the sample, the sum of tag abundance across each transcript (A_T) was computed, subtracted by A_{5ss}, and divided by the transcript length excluding the 6-nt-long 5′ SS region [i.e., $(A_T - A_{5ss})/(L - 6)$, where L is the transcript length].

Mapping of Transcription Start Sites. To analyze capped mRNA, 5′ P species in poly(A) RNA fraction were dephosphorylated with calf intestinal alkaline phosphatase (CIP) (New England Biolabs). The reaction was extracted with phenol/chloroform/IAA and then with chloroform and precipitated with ethanol. The reconstituted product was decapped with tobacco acid pyrophosphatase (TAP) (Epicentre) at 37 °C for 1 h. The reaction was extracted with phenol/chloroform/IAA and then with chloroform and precipitated with ethanol. A total of 100 ng of the resultant material was subject to 5′ RLM-RACE procedure as described above.

Northern Blot Analysis. Northern blot analysis was conducted as described previously (11). The *GLR1* mRNA was probed using oRP1659.

Half-Life Measurement. To measure mRNA decay rates, thiolutin (Sigma-Aldrich) was used to inhibit transcription. Cells were grown until OD_{600} of 0.4 was reached and treated with thiolutin at the final concentration of 3 μg/mL 7.5 mL of the cultures were collected at 0, 5, 10, 15, 30, and 60 min after transcription shutoff. Total RNA samples were analyzed by Northern blot analysis. The amount of *ERG13*, *CWP2*, *RPS31*, *RPS28B*, and 7S RNA was quantified by oRP1581, oRP1588, oRP1591, oRP1439, and oRP100, respectively, using Typhoon phosphoimager (Molecular Dynamics) and ImageQuant TL software (GE Healthcare). The mRNA levels were normalized to that of 7S RNA at each time point. Relative amount of mRNA at each time point compared with that at the 0-min time point was calculated and plotted as a function of the time the sample was collected after transcription shutoff. Half-lives were calculated by linear fitting of a semilog plot using Microsoft Excel. All time points were included for calculation of *CWP2* half-life in *dcp2*Δ and *dcp2*Δ *xrn1*Δ. Time points from 0 to 30 min were used otherwise. Two biological replicates were performed for each strain.

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10. Rissland OS, Norbury CJ (2009) Decapping is preceded by 3′ uridylation in a novel pathway of bulk mRNA turnover. *Nat Struct Mol Biol* 16:616–623.
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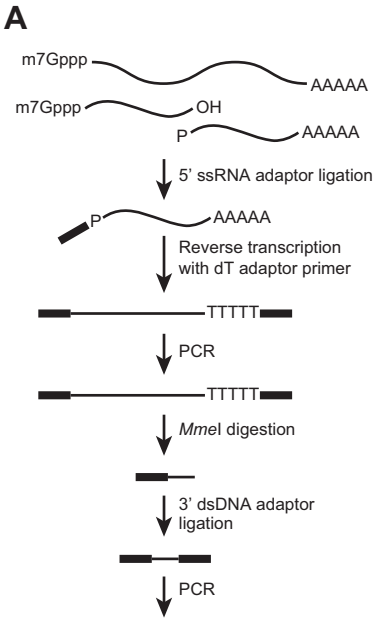

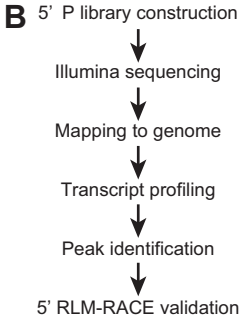

D

Replicates		Correlation
WT rep. 1	WT rep. 2	0.98
dcp2Δ xrn1Δ rep. 1	*dcp2Δ xrn1Δ* rep. 2	0.62
dcp2Δ xrn1Δ rep. 1	*dcp2Δ xrn1Δ* rep. 3	0.61
dcp2Δ xrn1Δ rep. 2	*dcp2Δ xrn1Δ* rep. 3	0.81

C

	WT rep. 1	WT rep. 2	*dcp2Δ xrn1Δ* rep. 1	*dcp2Δ xrn1Δ* rep. 2	*dcp2Δ xrn1Δ* rep. 3
Total tags	210,294,905 (100%)	58,087,979 (100%)	29,960,070 (100%)	170,828,378 (100%)	150,993,155 (100%)
20-21 nt tags	206,889,732 (93.4%)	56,123,272 (96.6%)	20,535,177 (68.5%)	164,170,232 (96.1%)	147,158,084 (97.5%)
Mapped to unique locations	156,900,727 (74.6%)	41,569,423 (71.6%)	14,935,945 (49.9%)	110,116,178 (64.5%)	122,680,892 (81.3%)
Mapped to mRNAs	142,023,128 (67.5%)	37,033,689 (63.8%)	11,692,191 (39.0%)	89,080,390 (52.2%)	79,557,271 (52.7%)

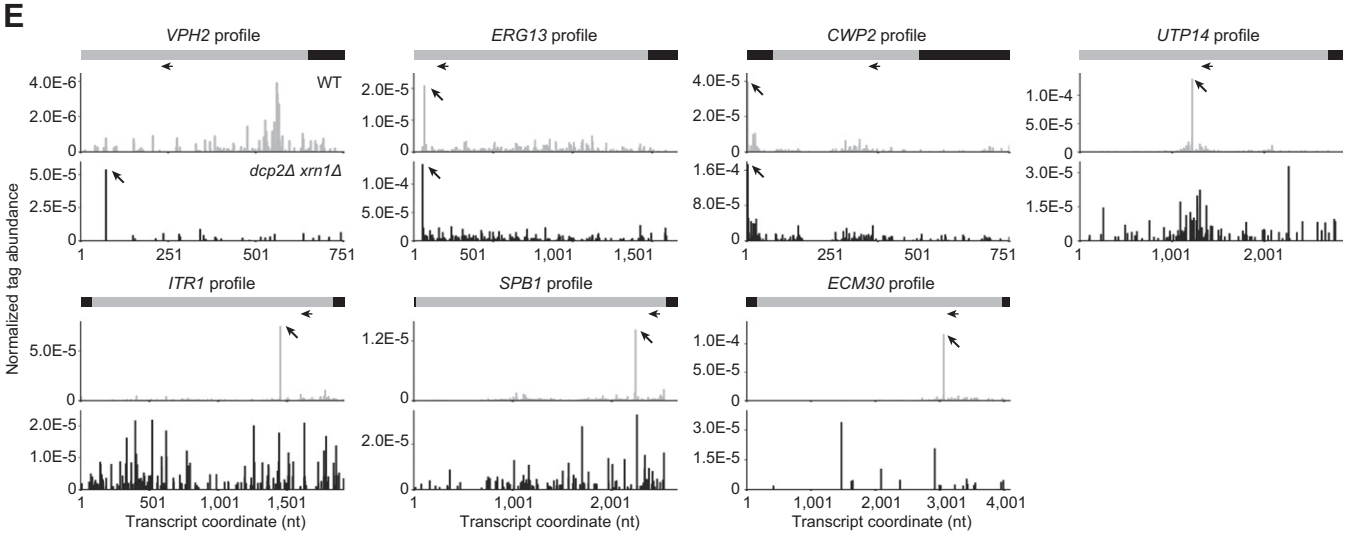

Fig. S1. Analysis of global 5′ P mapping data. (*A*) Schematic of the library construction. First, a 5′ RNA adaptor that introduces an MmeI recognition site was selectively ligated to 5′ P species in poly(A) RNA fraction. The ligated RNA was reverse-transcribed using oligo(dT) linked to a 3′ adaptor sequence, PCR-amplified, and treated with MmeI, which cleaves 20 or 21 nt 3′ of the recognition site. The product was ligated to 3′ dsDNA adaptor, PCR-amplified, and used for Illumina sequencing (SI Materials and Methods). (*B*) Outline of library analysis. (*C*) Summary statistics of high-throughput sequencing and mapping results. (*D*) Reproducibility of 5′ P signal at single-nucleotide resolution. For each pair of replicates, the Pearson correlation coefficient between raw tag abundance at each nucleotide in all mRNAs obtained for two replicates is shown. (*E*) The 5′ P tag profiles of *VPH2*, *ERG13*, *CWP2*, *UTP14*, *ITR1*, *SPB1*, and *ECM30*. Peaks are indicated by arrows. The x and y axes represent the same as in Fig. 2*B*. In the gene structures, UTRs and coding regions are indicated in black and light gray, respectively. Arrows under genes indicate locations of primers used in 5′ RLM-RACE.

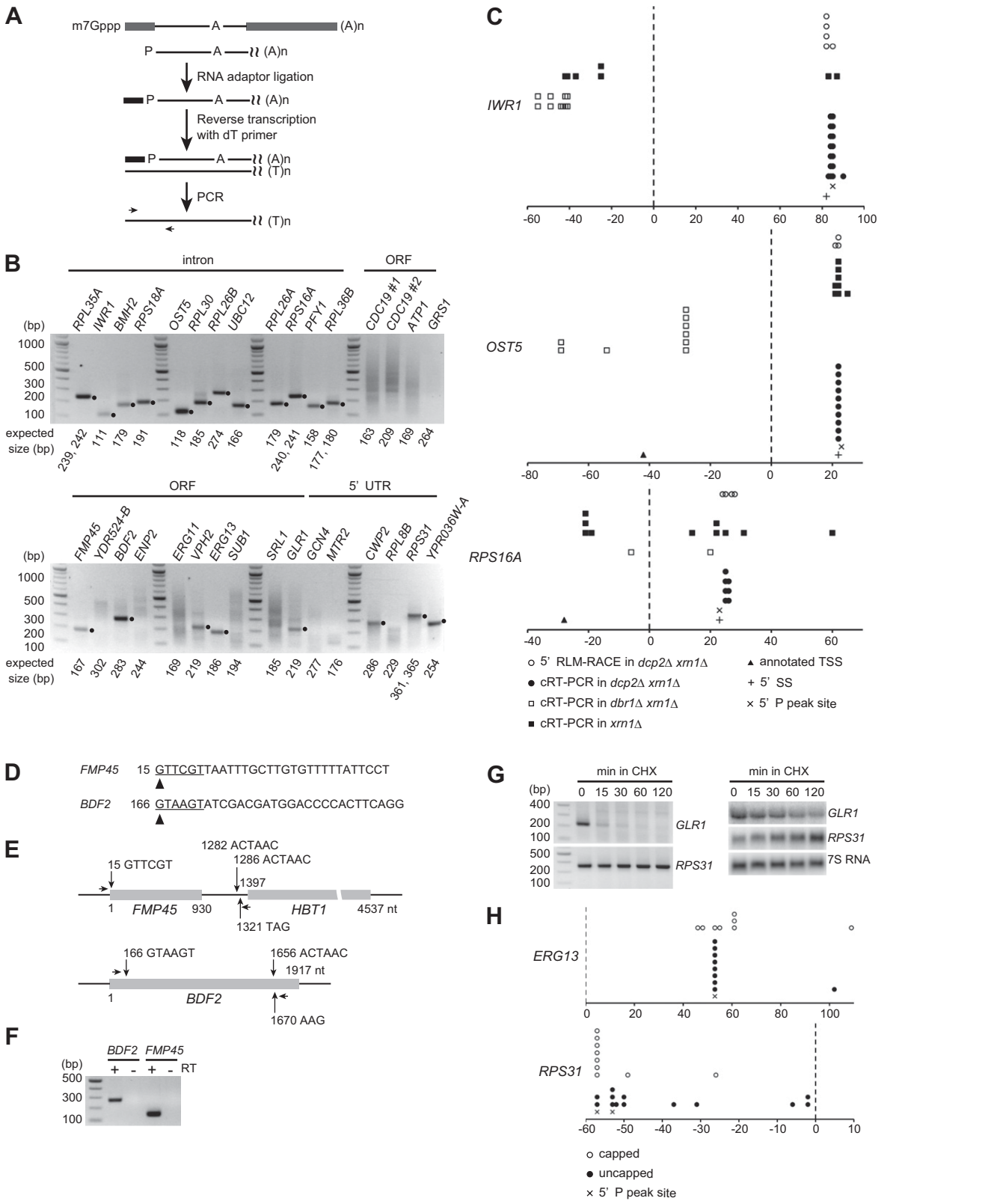

Fig. S2. Validation of 5′ P peaks by 5′ RLM-RACE and analysis of *FMP45*, *BDF2*, and *GLR1*. (*A*) Schematic of the gene-specific 5′ RLM-RACE procedure. The 5′ P population in poly(A) RNA fraction was ligated to a 5′ RNA adaptor primer oRP1522 and reverse transcribed using an oligo(dT) primer oRP1636. The resultant cDNA was PCR-amplified using a primer corresponding to the adaptor sequence (oRP1523) and a gene-specific reverse primer. Also see *SI Materials and Methods*. (*B*) Validation of 5′ P peaks in *dcp2*Δ *xrn1*Δ (yRP2860) by 5′ RLM-RACE. The expected size (bp) for amplicon was shown in the bottom of each lane.

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Closed circles indicate amplicons of the expected sizes. (*C*) Sequence of 5′ RLM-RACE and cRT-PCR products from *IWR1*, *OST5* and *RPS16A* genes. The 5′ ends of monophosphate species are plotted as a function of distance from start codons (0-based offset). Plus (+) and multiplication (×) signs represent positions of intron starts and tag peaks, respectively. Open circles represent 5′ P ends obtained by 5′ RLM-RACE. Closed circles and open and closed squares represent 5′ P ends obtained by cRT-PCR from *dcp2*Δ *xrn1*Δ, *dbr1*Δ *xrn1*Δ, and *xrn1*Δ strains, respectively. Some 5′ P ends from *IWR1* obtained by cRT-PCR were ambiguous because of the A nucleotide that could be derived either from genome or from poly(A) tail. For these ends, the two possible positions (+84 and +85) are plotted. Some 5′ P ends from *OST5* in *dbr1*Δ *xrn1*Δ and from *RPS16A* in *xrn1*Δ were mapped near the annotated transcription start sites (TSSs). (6) (closed triangles), consistent with these ends representing decapped unspliced pre-mRNAs. (*D*) The 5′ SS consensus sequences (underlined) at the 5′ tag peak sites (arrowhead) within *FMP45* and *BDF2*. (*E*) Schematic diagrams of the *FMP45* and *BDF2* loci. The 5′ and 3′ SSs revealed by sequencing of the amplicons in *F* and the consensus branch point sequences are shown. Arrows under genes indicate locations of primers used in *F*. (*F*) RT-PCR products from a *dcp2*Δ *xrn1*Δ strain. DNase-treated poly(A) RNA was reverse transcribed using with an oligo(dT) primer oRP1636. PCR was performed using primers depicted in *E* (oRP1647 and oRP1648 for *BDF2*; oRP1649 and oRP1650 for *FMP45*). (*G*) Translation is required for generation of 5′ P species from *GLR1*. Addition of cycloheximide in *GLR1* impaired the 5′ RLM-RACE signal from *GLR1* (*Center*) without substantially affecting the transcript level as shown by Northern blot (*Right*). A *glr1*Δ *xrn1*Δ strain (yRP2875) expressing the *GLR1* mRNA from pRP2407 was grown as described in *SI Materials and Methods*. (*H*) Sequence of capped and uncapped species in a *dcp2Δ xrn1Δ* strain (yRP2860). The 5′ ends of RACE products (Fig. 5*A*) are plotted as a function of distance from start codons (0-based offset). Multiplication (x) signs represent positions of tag peaks. Closed and open circles represent 5′ ends of uncapped and capped species, respectively.

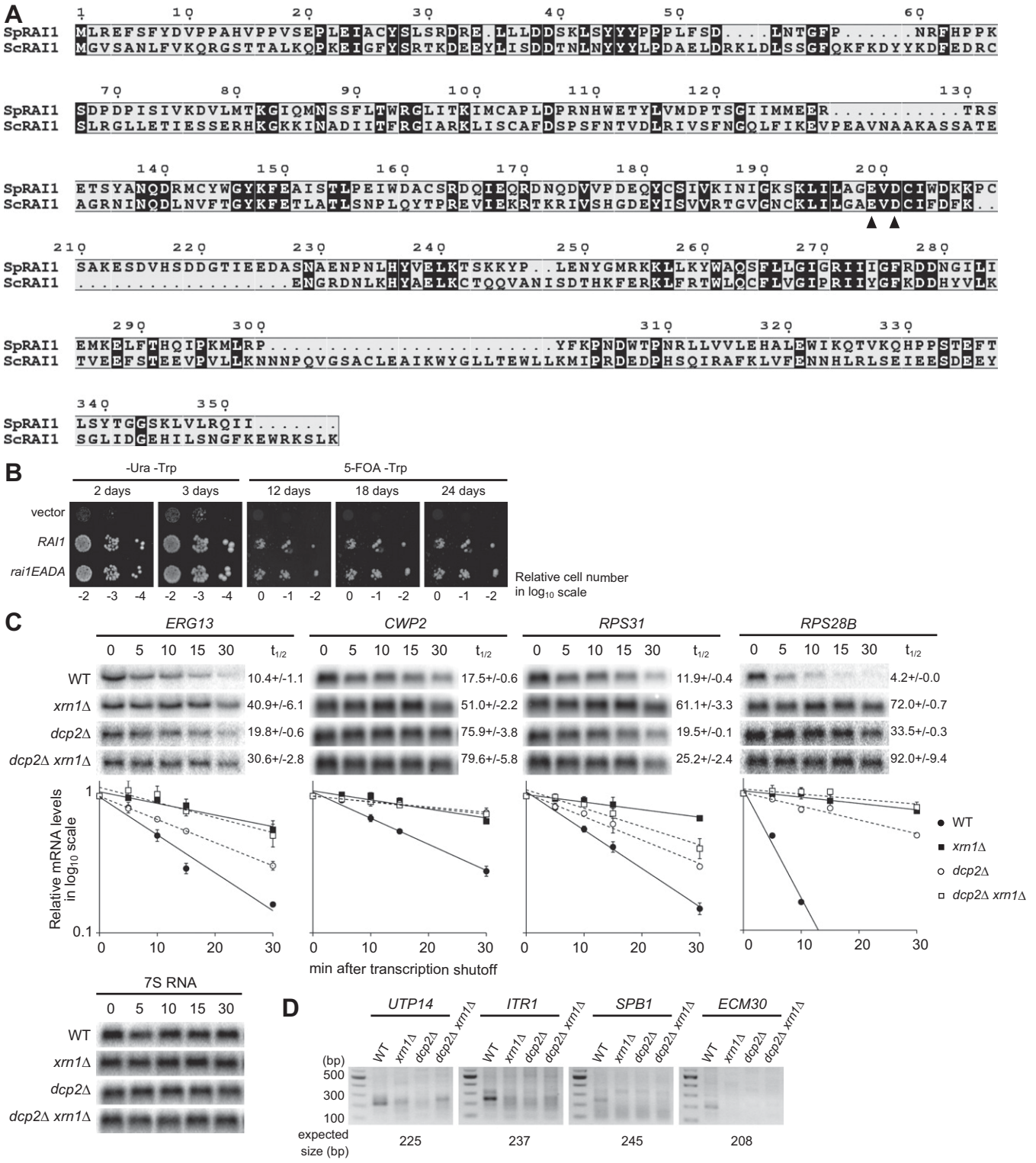

Fig. S3. Analysis of 5′ P ends located at transcription start sites. (*A*) Alignment of Rai1 proteins from *S. pombe* and *S. cerevisiae*. Arrowheads indicate residues that are critical for the catalytic activity. (*B*) Serial dilution spotting to show synthetic lethal interaction between *dcp2*Δ and *rai1*Δ. *dcp2*Δ *rai1*Δ strains carrying various *TRP1*-bearing plasmids in addition to a *URA3*-bearing plasmid expressing Dcp2(1-300)-GFP, which is sufficient to rescue the growth defect in *dcp2*Δ, were spotted onto either SC media lacking tryptophan and uracil but containing 2% (wt/vol) glucose or SC media lacking tryptophan but containing 2% (wt/vol) glucose and 5-Fluoroorotic Acid (5-FOA), which is counter selecting for *URA3*. The strain carrying an *TRP1*-bearing empty vector (yRP2869) was unable to grow in the absence of Dcp2 (1-300)-GFP (-Trp 5-FOA), whereas it formed colonies in its presence (-Trp -Ura), albeit slowly. The lethality and slow growth phenotypes were rescued by supplying a WT *RAI1* copy from a *TRP1*-bearing plasmid (yRP2870). The *rai1*EADA mutant (yRP2871) rescued these phenotypes as efficiently as WT, indicating that the catalytic activity is not critical for the ability of Rai1 to support normal growth rates. (*C*) Half-lives of *ERG13*, *CWP2*, *RPS31*, and *RPS28B* in WT, *xrn1*Δ, *dcp2*Δ, and *dcp2*Δ *xrn1*Δ strains (yRP2856, yRP2857, yRP2859, and yRP2860). Two biological replicates were performed for each strain. Representative Northern blot, average values and SDs of half-lives, semilog plots of relative mRNA levels against time points after transcription shutoff are shown. In the plots, error bars represent SDs. See *SI Materials and Methods*. (*D*) 5′ RLM-RACE for *UTP14*, *ITR1*, *SPB1*, and *ECM30* in WT, *xrn1*Δ, *dcp2*Δ, and *dcp2*Δ *xrn1*Δ strains (yRP2856, yRP2857, yRP2859, and yRP2860).

Table S1. Sequencing of 5′ RLM-RACE and cRT-PCR products from *IWR1*, *OST5*, and *RPS16A*

Gene	Method	Strain	5′ end	3′ end	No. clone
IWR1	5′ RLM-RACE	*dcp2*Δ *xrn1*Δ	+82	NA	4
			+85	NA	1
	cRT-PCR	*dcp2*Δ *xrn1*Δ	+83	+1,224	1
			+83	+1,229	1
			+84/85	+1,224	2
			+84/85	+1,232	1
			+84/85	+1,233	1
			+90	+1,222	1
		*dbr1*Δ *xrn1*Δ	−55	+1,224	1
			−55	+1,233	1
			−49	+1,229	1
			−44	+1,224	1
			−43	+1,224	1
			−42	+1,224	1
			−42	+1,233	1
			−41	+1,224	1
		*xrn1*Δ	−42	+1,224	1
			−41	+1,224	1
			−37	+1,203	1
			−25	+1,224	1
			−25	+1,229	1
			−83	+1,224	2
			−87	+1,224	1
OST5	5′ RLM-RACE	*dcp2*Δ *xrn1*Δ	+21	NA	1
			+22	NA	2
	cRT-PCR	*dcp2*Δ *xrn1*Δ	+22	+509	1
			+22	+555	1
			+22	+557	1
			+22	+558	7
		*dbr1*Δ *xrn1*Δ	−69	+548	1
			−69	+558	1
			−54	+509	1
			−28	+558	5
		*xrn1*Δ	+21	+541	1
			+21	+558	1
			+22	+546	1
			+22	+548	4
			+25	+558	1
RPS16A	5′ RLM-RACE	*dcp2*Δ *xrn1*Δ	+24	NA	1
			+25	NA	1
			+27	NA	1
			+28	NA	1
	cRT-PCR	*dcp2*Δ *xrn1*Δ	+25	+1,051	1
			+25	+1,062	2
			+25	+1,079	1
			+26	+1,062	2
			+26	+1,067	1
		*dbr1*Δ *xrn1*Δ	−6	+1,062	1
			+20	+1,077	1
		*xrn1*Δ	−21	+1,051	1
			−21	+1,055	1
			−21	+1,062	2
			−19	+1,065	1
			+14	+1,065	1
			+22	+1,048	1
			+22	+1,085	1
			+25	+1,062	1
			+31	+1,062	1
			+60	+1,085	1

The table shows termini of cloned 5′ RLM-RACE and cRT-PCR products in various strains. Nucleotide positions are indicated as distance from start codons (0-based offset).

Table S2. Yeast strains and plasmids used in this study

Name	Genotype/description	Source/reference
Yeast strains		
yRP2856 (W303-1A)	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	F. He and A. Jacobson
yRP2601 (W303-1B)	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	F. He and A. Jacobson
yRP2857	As W303-1A but with *xrn1Δ::kanMX*	This study
yRP2858	As W303-1B but with *xrn1Δ::kanMX*	This study
yRP2859	As W303-1A but with *dcp2Δ::HIS3*	F. He and A. Jacobson
yRP2860	As W303-1A but with *dcp2Δ::HIS3 xrn1Δ::kanMX*	This study
yRP2861	As W303-1A but with *dbr1Δ::hphMX*	This study
yRP2862	As W303-1B but with *dbr1Δ::hphMX*	This study
yRP2863	As W303-1A but with *dbr1Δ::hphMX xrn1Δ::kanMX*	This study
yRP2864	As W303-1A but with *dbr1Δ::hphMX dcp2Δ::HIS3*	This study
yRP2865	As W303-1A but with *dbr1Δ::hphMX dcp2Δ::HIS3 xrn1Δ::kanMX*	This study
yRP2866	As W303-1B but with *rai1Δ::hphMX*	This study
yRP2867	As W303-1A but with *dcp2Δ::HIS3 rai1Δ::hphMX xrn1Δ::kanMX pRS416-RAI1*	This study
yRP2868	As W303-1A but with *dcp2Δ::HIS3 rai1Δ::hphMX xrn1Δ::kanMX pRS416-rai1EADA*	This study
yRP2869	As W303-1A but with *dcp2Δ::HIS3 rai1Δ::hphMX pRS416-DCP2(aa1-300)-GFP pRS200*	This study
yRP2870	As W303-1A but with *dcp2Δ::HIS3 rai1Δ::hphMX pRS416-DCP2(aa1-300)-GFP pRS200-RAI1*	This study
yRP2871	As W303-1A but with *dcp2Δ::HIS3 rai1Δ::hphMX pRS416-DCP2(aa1-300)-GFP pRS200-rai1EADA*	This study
yRP2873	*MATa/alpha ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100 dcp2Δ::HIS3/DCP2 rai1Δ::hphMX/RAI1 xrn1Δ::kanMX/XRN1*	This study
yRP2874	As W303-1A but with *glr1Δ::HIS3MX*	This study
yRP2875	As W303-1A but with *glr1Δ::HIS3MX xrn1Δ::kanMX*	This study
yRP2876	As W303-1B with *dcs1Δ::hphMX*	This study
yRP2877	As W303-1A with *dcs1Δ::hphMX dcp2Δ::HIS3 xrn1Δ::kanMX*	This study
Plasmids		
pRS416	centromeric vector with the *URA3* marker	Lab stock
pRS200	centromeric vector with the *TRP1* marker	Lab stock
pSH47	pRS416 carrying the *GAL1* promoter, a sequence encoding the Cre recombinase, and the *CYC1* terminator	1
pRP1892	pRS416 carrying the *DCP2* promoter, a sequence encoding Dcp2(1-300)-GFP and the *ADH1* terminator	2
pRP2403	pRS416 carrying *RAI1*	This study
pRP2404	pRS416 carrying *rai1EADA*	This study
pRP2405	pRS200 carrying *RAI1*	This study
pRP2406	pRS200 carrying *rai1EADA*	This study
pRP2407	pRS416 carrying the *GAL1* promoter, a sequence encoding Glr1-Flag, and the *CYC1* terminator	This study
pRP2408	pRS416 carrying the *GAL1* promoter, a sequence containing a stem-loop RNA structure, a sequence encoding Glr1-Flag, and the *CYC1* terminator	This study

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2. Harigaya Y, Jones BN, Muhlrad D, Gross JD, Parker R (2010) Identification and analysis of the interaction between Edc3 and Dcp2 in Saccharomyces cerevisiae. *Mol Cell Biol* 30: 1446–1456.

Table S3. Oligonucleotides used in this study

Name	Sequence	Use/gene name	Reference
Strain construction			
oRP1655	ACTTGTAACAACAGCAGCAACAAATATATA TCAGTACGGTCGGATCCCCGGGTTAATTAA	To delete *XRN1*	This study
oRP1656	TAAAGTAACCTCGAATATACTTCGTTTTTAGTC GTATGTTGAATTCGAGCTCGTTTAAAC	To delete *XRN1*	This study
oRP1657	TGGCGTTCGCACAAGCGAAC	To confirm *XRN1* deletion	This study
oRP1658	CCCTTTGACAATCCCCATTTGTTATAAGCTTT	To confirm *XRN1* deletion	This study
oRP1606	GTAAATATGTAACTAAAAATTAAGATGGGCAGACATTTATCATT TTGCTTCGGATCCCCGGGTTAATTAA	To delete *DBR1*	This study
oRP1607	GGCTTGGCTTTAAGCTCTAATTCCGTCTGCATTCGTAATAGAAA TATCTCGAATTCGAGCTCGTTTAAAC	To delete *DBR1*	This study
oRP1608	AGATGTGTGTTTTTCTGGCCCT	To confirm *DBR1* deletion	This study
oRP1609	GGTCCAAAGGAAAGCACACATT	To confirm *DBR1* deletion	This study
oRP1610	GTAATATGGTGAAAGAATAGCGAAATATTAGACCAACATAGTGT ATCCCACGGATCCCCGGGTTAATTAA	To delete *RAI1*	This study
oRP1611	GATCCATACGTCGATGAGGATATGCGCAGGAAAGACATAAAGGAA TATTGGAATTCGAGCTCGTTTAAAC	To delete *RAI1*	This study
oRP1612	GGCATCTTGATCCCTTCTATGC	To confirm *RAI1* deletion	This study
oRP1613	ACGTTGCAATGAATGGTCAAAA	To confirm *RAI1* deletion	This study
oRP1614	AGTCACATCAAGATCGTTTATGG	To check mating type	1
oRP1615	GCACGGAATATGGGACTACTTCG	To check mating type	1
oRP1616	ACTCCACTTCAAGTAAGAGTTTG	To check mating type	1
Plasmid construction			
oRP1617	GCAAGTTAATTCTTGGTGCTGCTGTGGCTTGTATATTTGACTTTAAAGA	To generate pRP2404 and pRP2406	This study
oRP1618	TCTTTAAAGTCAAATATACAAGCCACAGCAGCACCAAGAATTAACTTGC	To generate pRP2404 and pRP2406	This study
oRP1619	CGGGAGCTCGACTTCACCCATTTTGGCATCT	To generate pRP2403-2406	This study
oRP1620	CCGCTCGAGGGAGCATAACGTGAGGGAAGAA	To generate pRP2403-2406	This study
oRP1660	TATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCTAGAACAG AACTTTATGCTTTCTGCAACCA	To generate pRP2407	This study
oRP1661	CGTGAATGTAAGCGTGACATAACTAATTACATGACTCGAGTCAC TTGTCATCGTCGTCCTTGTAGTCTCTCATAGTAACCAATTCTTCTGCG	To generate pRP2407 and pRP2408	This study
oRP1662	TATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCTAGAGAT CCCGCGGTTCGCCGCGGACAGAACTTTATGCTTTCTGCAACCA	To generate pRP2408	This study
Library construction			
oRP1627	rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArC	5′ RNA adaptor	2
oRP1628	CGAGCACAGAATTAATACGACTTTTTTTTTTTTTTTTTV	oligo(dT) adaptor primer	2
oRP1629	GTTCAGAGTTCTACAGTCCGAC	PCR primer	2
oRP1630	CGAGCACAGAATTAATACGAC	PCR primer	2
oRP1631	P-TCGTATGCCGTCTTCTGCTTG	3′ dsDNA adaptor top	2
oRP1632	CAAGCAGAAGACGGCATACGANN	3′ dsDNA adaptor bottom	2
oRP1633	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA	P5 primer	2
oRP1634	CAAGCAGAAGACGGCATACGA	P7 primer	2
oRP1635	CCACCGACAGGTTCAGAGTTCTACAGTCCGAC	Sequencing primer	This study
5′ RLM-RACE			
oRP1522	rGrCrUrGrArUrGrGrCrGrArUrGrArArUrGrArArCrArCrUr GrCrGrUrUrUrGrCrUrGrGrCrUrUrUrGrArUrGrArArA	5′ RNA adaptor	3
oRP1636	TTTTTTTTTTTTTTTTV	oligo(dT) RT primer	This study
oRP1523	CTGATGGCGATGAATGAACACT	Adaptor primer	3
oRP1637	GGTTCTGGTATGTGTAAAGCCG	Forward PCR primer for *ACT1*	This study
oRP1638	TCTTGGATTGAGCTTCATCACC	Reverse PCR primer for *ACT1*	This study
To detect peaks in introns			
oRP1556	CCTTGATTCTTTGCTTGCGAAT	*RPL35A*	This study
oRP1557	CTGAATTGTCCCAGATGAACTTAG	*IWR1*	This study
oRP1558	GCTTTTCCCCCTTAATCAATCG	*BMH2*	This study
oRP1559	CAGCCCGGGAACATCATTATTA	*RPS18A*	This study
oRP1560	GGGAACTATTCATTGTCCTGTGTG	*OST5*	This study
oRP1561	ATGGAGAAGCATCCTTTGAACG	*RPL30*	This study
oRP1562	TGCTAAATAGTCGCCCTATGCC	*RPL26B*	This study
oRP1563	AGCCAAGAATATACATATCCAGTACACA	*UBC12*	This study
oRP1564	CAACCTTGGATAACGCACCTT	*RPL26A*	This study
oRP1565	CTCTCCCCAAAACATTGCCTAA	*RPS16A*	This study
oRP1566	AATCTTCGAAATTACGCGCATC	*PFY1*	This study
oRP1567	ACGCTGGGTGCAGGATATAAAC	*RPL36B*	This study
To detect peaks in ORFs			
oRP1568	CAAGTCACCTCTGGCAACCATA	*CDC19*	This study
oRP1569	TACCGAAGTTGATACGGGCTTC	*CDC19*	This study

Table S3. Cont.

Name	Sequence	Use/gene name	Reference
oRP1570	CCAAGTATTGTAGAGGAGCGGC	*ATP1*	This study
oRP1571	TTTCTGAATTTTGGACCGAACA	*GRS1*	This study
oRP1572	GCGCTGTTAAAGCCGTTTGTAT	*FMP45*	This study
oRP1575	GGTAGTTCCTCCGCTTCCATTT	*BDF2*	This study
oRP1577	TTTCCTTCTTTGGTCCCTTCAA	*YDR524C-B*	This study
oRP1578	TAAAACGTTCGCTTTGCTCCTT	*ENP2*	This study
oRP1579	ACATCTGTGTCTACCACCACCG	*ERG11*	This study
oRP1580	GTGTAAACCTCTGACGGCCTTG	*VPH2*	This study
oRP1582	TTGGTTGACACATTGAGTTGGG	*ERG13*	This study
oRP1583	CTTCTTCTTTGAACGTGGCTCC	*SUB1*	This study
oRP1584	TGCACCGGTGACAGTAACGTAT	*SRL1*	This study
oRP1585	GGGATTTACGTCCAATTGTCCA	*GLR1*	This study
oRP1673	GGTTTCAGCGTCATTTGTCATACCAT	*UTP14*	This study
oRP1674	GATAAGCACGACAATCCCGCGAAA	*ITR1*	This study
oRP1675	CCTGCCAAACCTTTGTTCCTACCT	*SPB1*	This study
oRP1676	GAGATTCAAATTGCCTCAATTCATGCGA	*ECM30*	This study
To detect peaks in 5′ UTRs			
oRP1586	AACCATCCAATGGTGAGAAACC	*GCN4*	This study
oRP1587	TTACTTGCTCTATGCGTTTGCG	*MTR2*	This study
oRP1588	ATAGTTTCGGTGCTGGATGGAG	*CWP2*	This study
oRP1590	TTCTGGCCATTTGACGTATCTG	*RPL8B*	This study
oRP1591	TGGGGTGGTGTAGACCTTCTTC	*RPS31*	This study
oRP1592	GAAGCAGAGGCATCCAAAAGAG	*YPR036W-A*	This study
cRT-PCR			
oRP1557	CTGAATTGTCCCAGATGAACTTAG	RT primer for *IWR1*	This study
oRP1639	GTCAAACGCATTTTTGTTATATCGGT	PCR primer R1 for *IWR1*	This study
oRP1640	ATGTTGATGACCCATTGGCG	PCR primer F1 for *IWR1*	This study
oRP1560	GGGAACTATTCATTGTCCTGTGTG	RT primer for *OST5*	This study
oRP1641	AAAGAATAGCATCAACCAGCGT	PCR primer R1 for *OST5*	This study
oRP1642	GTGGCTCTTCGTTGGCCTATAA	PCR primer F1 for *OST5*	This study
oRP1565	CTCTCCCCAAAACATTGCCTAA	RT primer for *RPS16A*	This study
oRP1643	TTCTCTTGATCTCACTCCATTATCG	PCR primer R1 for *RPS16A*	This study
oRP1644	GTGGTAAGGGTGCTCGTTCC	PCR primer F1 for *RPS16A*	This study
oRP1645	CGGTGATTTCCTTTTGCATT	RT primer for *ACT1*	This study
oRP1637	GGTTCTGGTATGTGTAAAGCCG	Forward PCR primer for *ACT1*	This study
oRP1638	TCTTGGATTGAGCTTCATCACC	Reverse PCR primer for *ACT1*	This study
RT-PCR			
oRP1647	CAAGACACGCACATTCTGCTTT	Forward PCR primer for *BDF2*	This study
oRP1648	GTTGGCGCAGTCGAGTAGTTG	Reverse PCR primer for *BDF2*	This study
oRP1649	GAAAGAGGTATTTACGTTTAACGC	Forward PCR primer for *FMP45*	This study
oRP1650	GCTCTCCACTGGGAAATCTTCA	Reverse PCR primer for *FMP45*	This study
Northern blot analysis			
oRP1659	CTTTATAGCAACACCGAACCCTTGCA	To probe *GLR1*	This study
oRP1581	GGCCCAGACCAATTGTGTATTT	To probe *ERG13*	This study
oRP1588	ATAGTTTCGGTGCTGGATGGAG	To probe *CWP2*	This study
oRP1591	TGGGGTGGTGTAGACCTTCTTC	To probe *RPS31*	This study
oRP1439	CATCATTGAGTATTTCTACGCATTTG	To probe *RPS28B*	4
oRP100	GTCTAGCCGCGAGGAAGG	To probe 7S RNA	5

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3. Chen L, et al. (2010) Structure of the Dom34-Hbs1 complex and implications for no-go decay. *Nat Struct Mol Biol* 17:1233–1240.
4. Harigaya Y, Jones BN, Muhlrad D, Gross JD, Parker R (2010) Identification and analysis of the interaction between Edc3 and Dcp2 in Saccharomyces cerevisiae. *Mol Cell Biol* 30: 1446–1456.
5. Caponigro G, Muhlrad D, Parker R (1993) A small segment of the MAT alpha 1 transcript promotes mRNA decay in Saccharomyces cerevisiae: A stimulatory role for rare codons. *Mol Cell Biol* 13:5141–5148.

Dataset S1. List of 6,603 protein-coding transcripts used in 5′ P profiling

Dataset S1

Dataset S2. List of 5′ P peak sites. (*A*) List of 5′ P peak sites in the WT library. The table contains 2,738 sites identified with the Benjamini–Hochberg false discovery rate (FDR) procedure (FDR <0.01). (*B*) List of 5′ P peak sites in the *dcp2*△ *xrn1*△ library. The table contains 153,593 sites identified with the Benjamini–Hochberg FDR procedure (FDR <0.01). (*C*) 5′ RLM-RACE validation of selected sites. Plus signs (+) indicate that a PCR amplicon of the expected size was obtained, and minus signs (–) indicate otherwise.

Dataset S2