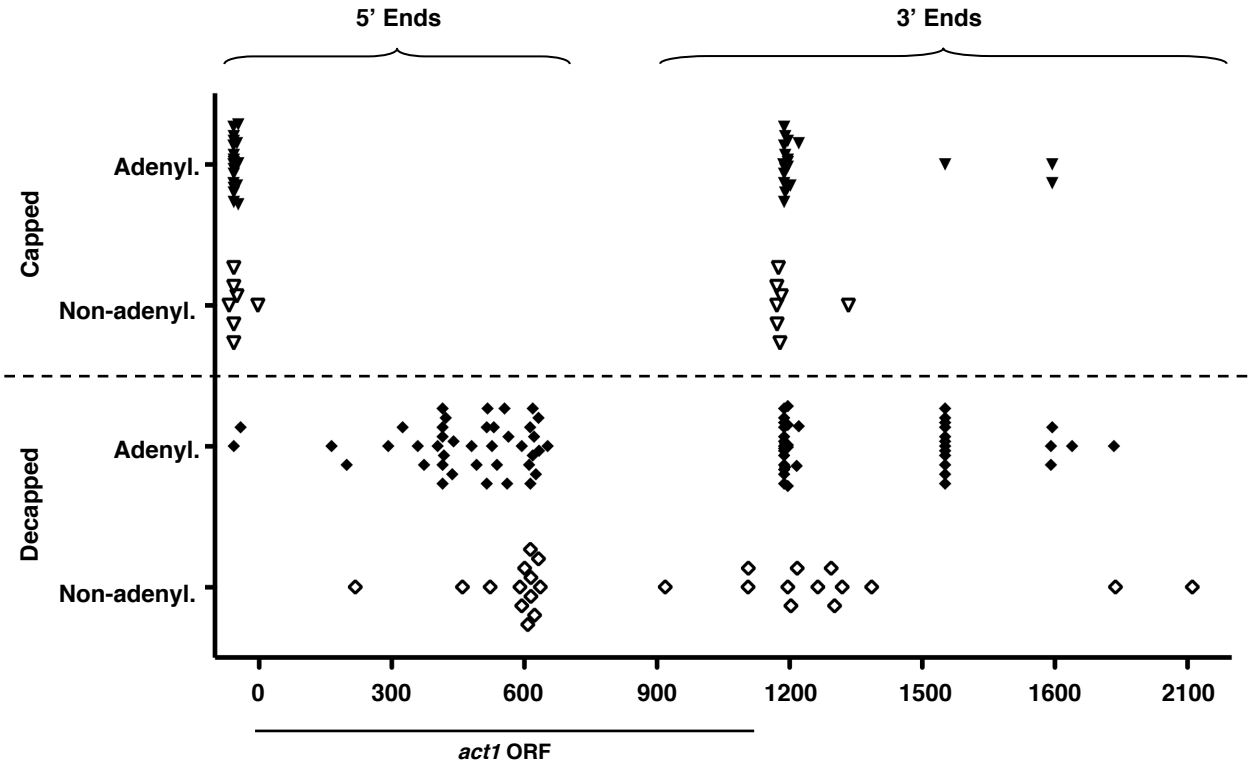
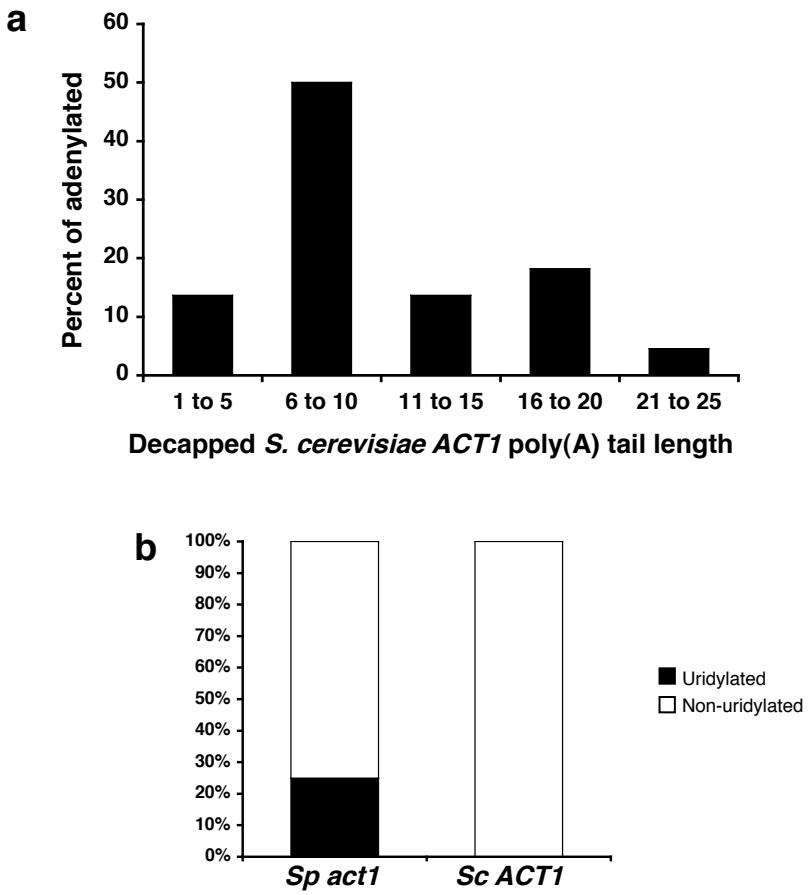
3′ uridylation precedes decapping in a novel pathway of bulk mRNA turnover

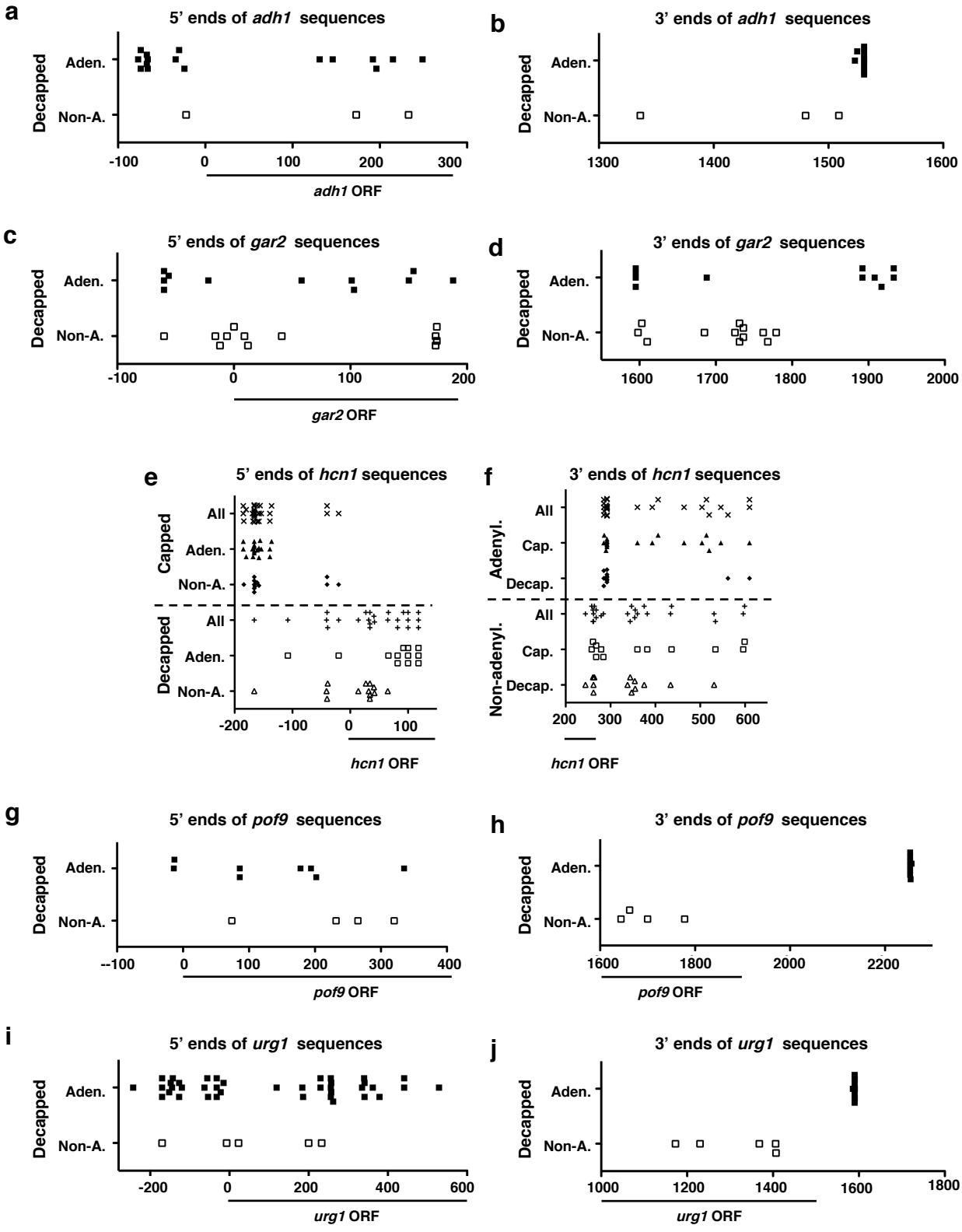
Olivia S. Rissland and Chris J. Norbury



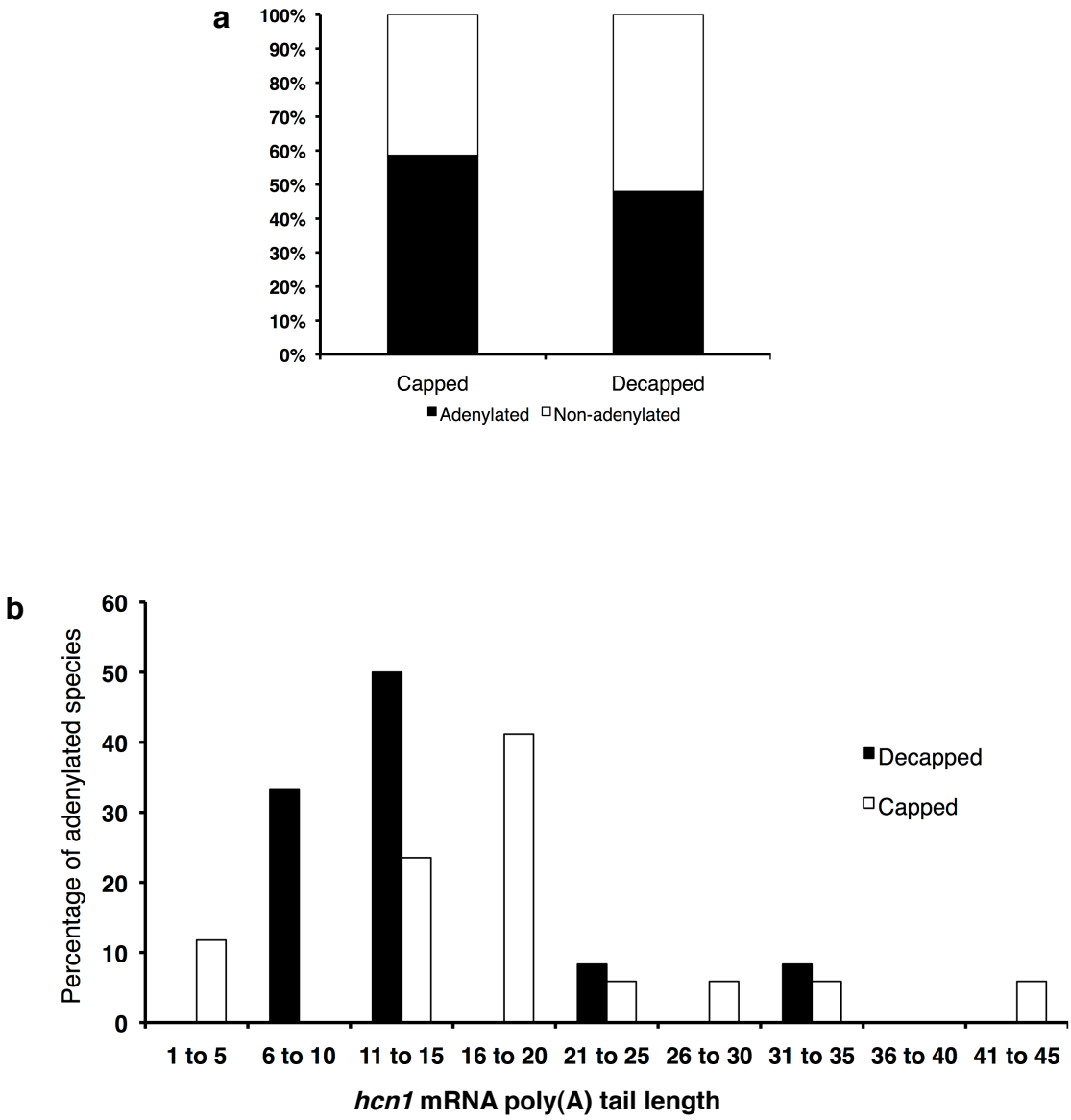
Supplementary Figure 1. *act1* cRACE sequences from WT cells. The 5′ and 3′ ends of various types of *act1* cRACE sequences are plotted as the distance (in nt, as indicated on the horizontal axis) from the start codon. The open reading frame (ORF) is marked with a line.



Supplementary Figure 2. In *S. cerevisiae*, *ACT1* mRNA is degraded by deadenylation-dependent mechanisms. (**a**) Poly(A) tail lengths of decapped *S. cerevisiae ACT1* messages were binned into groups of ten nt. Tail lengths were then plotted as percentage of adenylated species. (**b**) The percentage of decapped, adenylated transcripts that contain [in black] or lack [in white] a terminal uridyl residue is plotted for *act1* messages isolated from *S. pombe* (n=40) or *S. cerevisiae* cells (n=22).

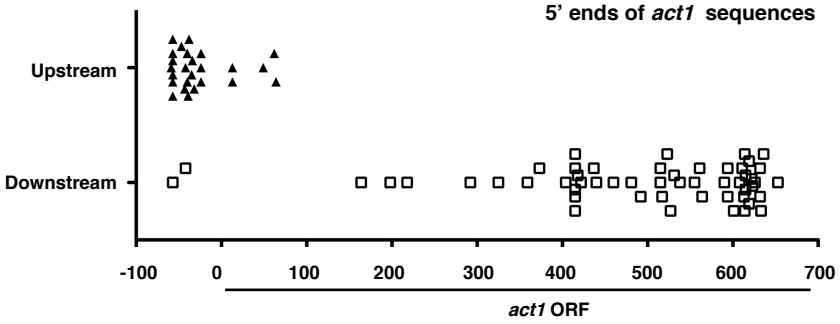


Supplementary Figure 3. *adh1*, *gar2*, *hcn1*, *pof9* and *urg1* cRACE sequences from WT cells. The 5′ and 3′ ends of various types of *adh1* (**a**,**b**), *gar2* (**c**, **d**), *hcn1* (**e**, **f**), *pof9* (**g**, **h**) and *urg1* (**I**, **j**) cRACE sequences are plotted as the distance (in nt, as indicated on the horizontal axis) from the start codon. Where appropriate, the open reading frame (ORF) is marked with a line.



Supplementary Figure 4. *hcn1* mRNA is degraded mainly by deadenylation-dependent decay. (**a**) The percentage of adenylated [black] and nonadenylated [white] sequences for capped (n=18) and decapped (n=12) *hcn1* transcripts is shown. (**b**) Poly(A) tail lengths of decapped [black] and capped [white] sequences were binned into groups of five nt. Tail lengths were then plotted as percentage of adenylated species.

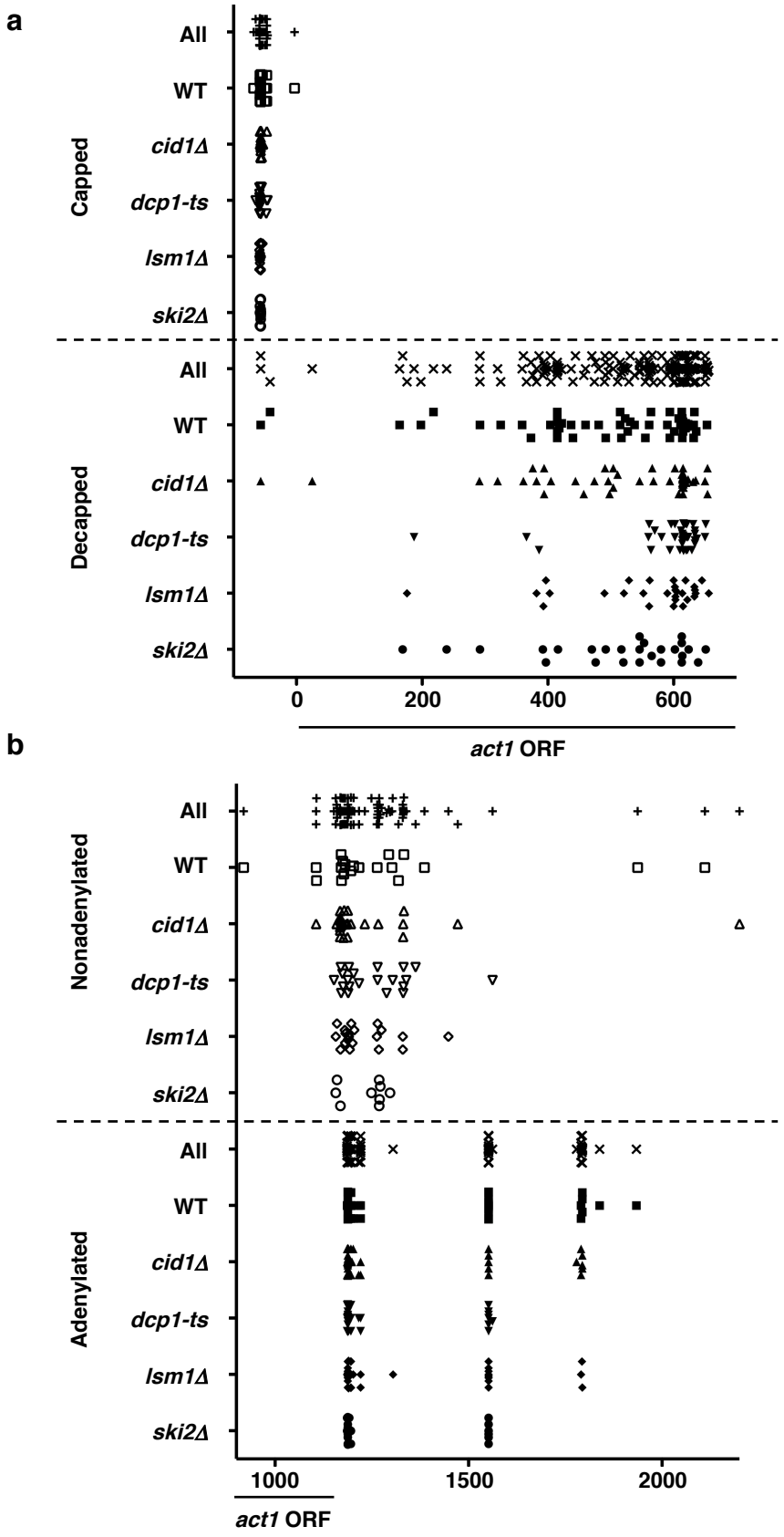
a



b

Strain	Primer Location	Number Uridylated	Total	Percentage Uridylated
Wild-type	Downstream	10	40	25.0%
	Upstream	3	14	21.5%
ski2Δ	Downstream	5	18	27.8%

Supplementary Figure 5. Uridylation precedes extensive 5' – 3' mRNA degradation. (**a**) The 5′ ends of *act1* cRACE sequences, generated from either the upstream or downstream primer, are plotted as the distance (in nt, as indicated on the horizontal axis) from the start codon. The open reading frame (ORF) is marked with a line. (**b**) The percentages of uridylated sequences in cRACE products generated using the downstream (n=40) and upstream (n=14) *act1* primers are shown, as well as that observed in *ski2Δ* cells.

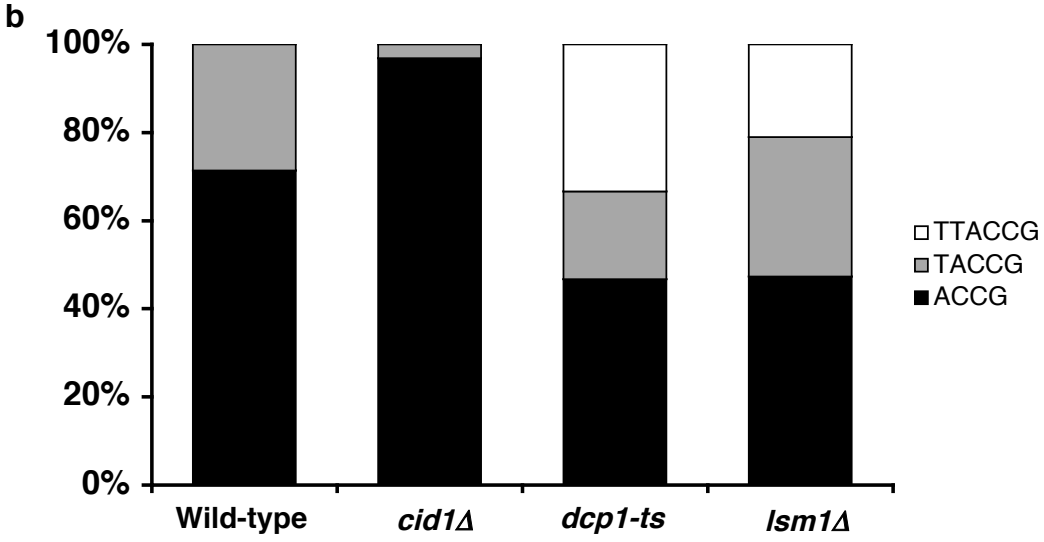


Supplementary Figure 6. *act1* cRACE products from various strains. The 5′ (**a**) and 3′ (**b**) ends of various types of *act1* cRACE sequences from WT, *cid1Δ*, *dcp1-ts*, *lsm1Δ* and *ski2Δ* are plotted as the distance (in nt, as indicated on the horizontal axis) from the start codon. The open reading frame (ORF) is marked with a line.

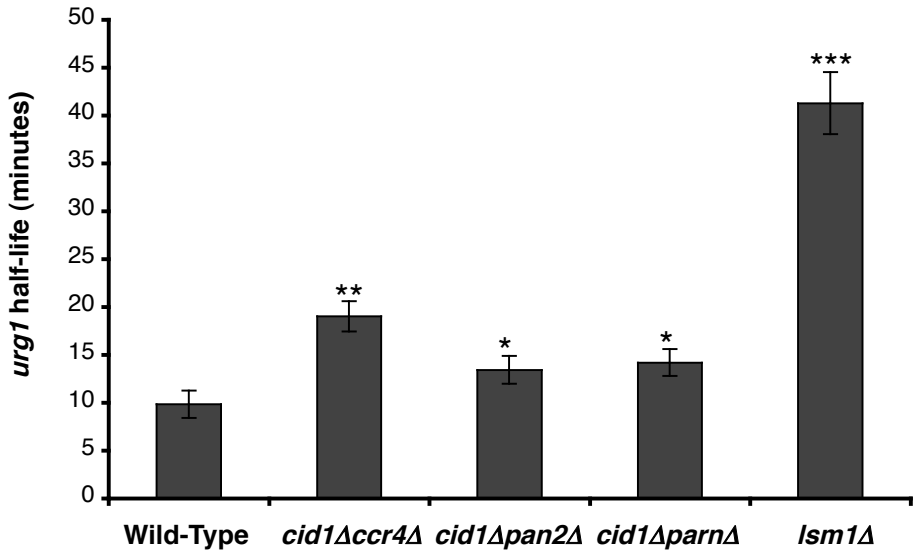
a

5′ End	Uridylated?	Expected cRACE product
-57	No	….AAAAAAA**ACCG**
-57	Yes	….AAAAAAT**ACCG**
-58	No	….AAAAAA**TACCG**
-58	Yes	….AAAAAT**TACCG**
-59	No	….AAAAA**TTACCG**
-59	Yes	….AAAAT**TTACCG**

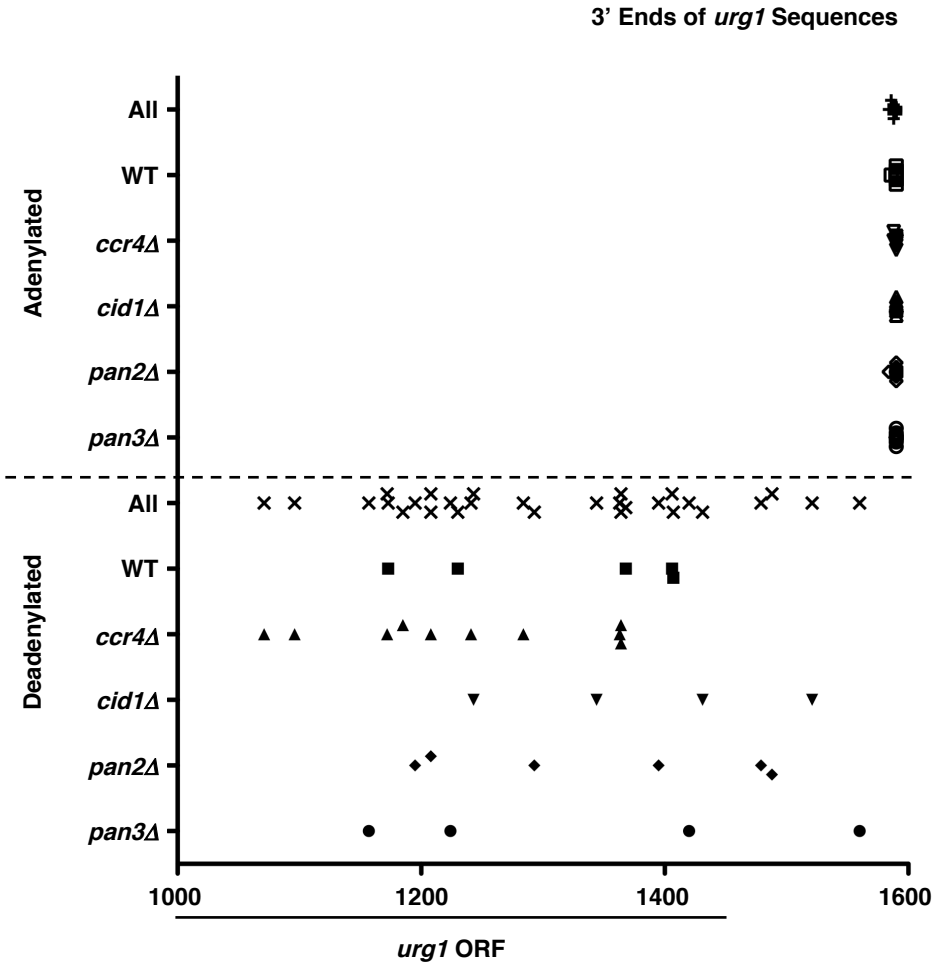
b



Supplementary Figure 7. Uridylated, capped *act1* mRNAs accumulate in *dcp1-ts* and *lsm1Δ* cells. (a) Due to the position of the most abundant transcriptional start site of *act1*, it was not possible to definitively determine whether capped mRNA was uridylated. That is, in the DNA sequence, the main start site (at −57 bases) is preceded by two thymines: a capped mRNA starting at position −57 and containing a terminal uridyl residue is therefore indistinguishable from a non-uridylated transcript starting at nucleotide −58. Similarly, a capped mRNA, starting at position –57, containing two terminal uridyl residues is indistinguishable from a non-uridylated transcript starting at position –59. A table of these possibilities is presented along with the expected cRACE product: nucleotides underlined denote those derived from the 3′ tail; those in bold denote those derived from the 5′ end. (b) If 3′ terminal uridyl residues had been incorrectly assigned to the 5′ end, conditions that decrease uridylation might result in an apparent shift in the transcription start site to the −57 position and those that increase might result in an apparent shift to the −58 and −59 positions. Of those transcripts appearing to initiate at the –57, –58 or –59 positions, the percentage of these sites is plotted [–59 position or TTACCG in white; –58 position or TACCG in grey; –57 or ACCG in black] for capped *act1* transcripts isolated from WT cells (14 samples), *cid1Δ* cells (32 samples), *dcp1-ts* cells (15 samples) or *lsm1Δ* cells (19 samples). Consistent with this interpretation, there was a significant, apparent shift to the –57 position in *cid1Δ* cells when compared with WT cells (p=0.005). In *dcp1-ts* and *lsm1Δ* cells, there was a significant, apparent shift in start site usage to the –58 and –59 positions in comparison to WT cells (p=0.02 and p=0.03, respectively).



Supplementary Figure 8. *urg1* mRNA is stabilized in *cid1Δccr4Δ*, *cid1Δpan2Δ*, *cid1ΔparnΔ* and *lsm1Δ* cells. Cells were harvested at 0, 10, 20 and 30 minutes after uracil washout. After probing for *urg1* and *pik1* by northern blotting, *urg1* levels were normalized to *pik1* mRNA. The percent of *urg1* remaining at each time point was calculated by a comparison to the normalized amount at 0 minutes. The half-life of *urg1* in different strains is shown. At least two independent replicates were performed for each strain. * denotes $p<0.05$; ** denotes $p<0.001$; *** denotes $p<0.0005$. Error bars represent standard deviation.



Supplementary Figure 9. *urg1* cRACE sequences from various strains. The 3′ ends of various types of *urg1* cRACE sequences from WT, *ccr4Δ*, *cid1Δ*, *pan2Δ* and *pan3Δ* are plotted as the distance (in nt, as indicated on the horizontal axis) from the start codon. The open reading frame (ORF) is marked with a line.

Supplementary Table 1. *act1* HSC-RACE products from wild-type cells.

Clone	*3′ End*	*Tail*
ASC01	…TCTTCTGATATA (+1190)	$(A)_{13}$
ASC02	…ATTCTTCTGATA (+1188)	$(A)_{17}U$
ASC03	…ATTCTTCTGATA (+1188)	$(A)_{21}$
ASC04	…ATATATATAAA (+1196)	$(A)_{27}G(A)_{8}$
ASC05	…ATTCTTCTGATA (+1188)	$(A)_{35}$
ASC13	…CGATTCTTCTGA (+1186)	$(A)_{19}$
ASC15	…TCTTCTGATATA (+1190)	$(A)_{15}$
ASC16	…TCTTCTGATATA (+1190)	$(A)_{25}U$
ASC17	…ATTCTTCTGATA (+1188)	$(A)_{16}$
ASC19	…ATTCTTCTGATA (+1188)	$(A)_{12}GA$
ASC20	…ATTCTTCTGATA (+1188)	$(A)_{30}$
ASC21	…ATTCTTCTGATA (+1188)	$(A)_{11}UCA$
ASC22	…ATTCTTCTGATA (+1188)	$(A)_{7}$
ASC24	…TGAGGAACTTTG (+1260)	No Tail
ASC25	…CGATTCTTCTGA (+1186)	$(A)_{14}$
ASC26	…ATTCTTCTGATA (+1188)	$(A)_{18}U$
ASC27	…TCTTCTGATATA (+1190)	$(A)_{13}$
ASC28	…ATTTCAATCTTT (+1211)	No Tail
ASC30	…ATTCTTCTGATA (+1188)	$(A)_{38}$
ASC31	…TCTTCTGATATA (+1190)	$(A)_{28}$
ASC34	…ATATATATAAA (+1196)	$C(A)_{36}$

Supplementary Table 2. *act1* HSC-RACE products from *dcp1-ts* cells.

Clone	*3′ End*	*Tail*
AdSC01	…TTTGTAACGTTT (+1152)	No Tail
AdSC02	…ACATACTTTTGA (+1167)	No Tail
AdSC03	…ATATATATAAA (+1196)	$(A)_5U$
AdSC04	…ATTCTTCTGATA (+1188)	$(A)_{13}UU$
AdSC05	…GTAACGTTTTTT (+1155)	No Tail
AdSC06	…TTCAATCTTTTT (+1209)	No Tail
AdSC07	…ATTCTTCTGATA (+1188)	$(A)_{14}UU$
AdSC08	…TCTTCTGATATA (+1190)	$(A)_4U$
AdSC09	…ACTTTTTGATTC (+1148)	No Tail
AdSC10	…ATTCTTCTGATA (+1188)	$(A)_{20}$
AdSC14	…CGATTCTTCTGA (+1186)	$(A)_{14}U$
AdSC15	…TATAAATTTCAA (+1202)	$(A)_{26}$
AdSC16	…ATATATATAAA (+1196)	$(A)_6UU$
AdSC17	…CGATTCTTCTGA (+1188)	$(A)_{12}U$
AdSC18	…CGATTCTTCTGA (+1186)	$(A)_{18}$
AdSC19	…TTCAATCTTTTT (+1209)	No Tail
AdSC20	…TATAAATTTCAA (+1202)	$(A)_{26}$
AdSC21	…ATATATATAAA (+1196)	$(A)_8UU$
AdSC22	…CGATTCTTCTGA (+1188)	$(A)_8(U)_4$
AdSC23	…ATATATATAAA (+1196)	$(A)_7U$
AdSC25	…CGATTCTTCTGA (+1188)	$(A)_{10}UU$
AdSC26	…ATATATATAAA (+1196)	$(A)_8U$
AdSC27	…CGATTCTTCTGA (+1188)	$(A)_{11}U$
AdSC28	…TTCAATCTTTTT (+1209)	No Tail
AdSC29	…TATAAATTTCAA (+1202)	$(A)_7$
AdSC30	…TTCTGATATATA (+1190)	$(A)_{10}UC(U)_4$
AdSC31	…TCTTCTGATATA (+1190)	$(A)_{19}$

Supplementary Table 3. Oligonucleotides used in this study.

Name	*Sequence (5′ to 3′)*	*Use*
M13 Reverse Primer	CAGGAAACAGCTATGAC	Sequencing
M13 Forward Primer	GTAAAACGACGGCCAG	Sequencing
5' T7 Actin	TAATACGACTCACTATAGGGAGGAAAGTAGAAAGAGAAG	HSC-RACE RNA probe; cRACE 1st PCR primer
3' T7 Actin	CCACTATGTATCCCGGTATTGC	HSC-RACE RNA probe
RNase H Actin	CTCATCATACTCTTGCTTGG	HSC-RACE
Seq Actin	GCTCCTCTTACTTTTTGTAACG	HSC-RACE; cRACE 2nd PCR primer
RT Actin	GAAGCACTTACGGTAAACGATAC	HSC-RACE
Upstream 5' T7 Actin	TAATAGACTCACTATAGGGACTCAAAGTCCAAAGCGAC	cRACE RT primer
HCN1 RT 1	CCAATCCTTCTTTCAC	cRACE RT, 1st PCR primer
HCN1 RT 2	CTTTTCAGGCGATAAAC	cRACE 2nd PCR primer
HCN1 PCR 1	CTTCAATGCATCCTTCCC	cRACE RT, 1st PCR primer
HCN1 PCR 2	GGTGGAGCCGCTCAATCATAG	cRACE 2nd PCR primer
Urg1 RT 2	CGGCAACAGCTAAAGC	cRACE 2nd PCR primer
Urg1 RT 1	GGTGGGACGAACGTCG	cRACE 1st PCR primer; Northern blot probe primer
Urg1 PCR 1	CA AATACCTTGT TTACAAC	cRACE 1st PCR primer
Urg1 PCR 2	GAAGAGTTGGCCGGTGTTCG	cRACE 2nd PCR primer
Actin cRACE Upstream	GAGGGGAATACAGCTCTAG	Upstream cRACE primer
Sc Act1 RT1	CGTTGTAGAAGGTATGATGCCAGATC	cRACE RT, 1st PCR primer
Sc Act1 RT2	CCAGTTGGTGACAATACCGTGTTC	cRACE 2nd PCR primer
Sc Act1 PCR1	GTGATGTCGATGTCCGTAAGG	cRACE 1st PCR primer
Sc Act1 PCR2	GGTGGTACCACCATGTTCCCAGG	cRACE 2nd PCR primer
adh1 RT 2	GGTCACCAATCTTAAGACG	cRACE 2nd PCR primer
adh1 RT 1	CAGTACTCGCAGTTACCGC	cRACE 1st PCR primer
adh1 PCR 1	GATCCACTTTTAATTCCTAATG	cRACE 1st PCR primer
adh1 PCR 2	CTTTTACCATTTCACCACAC	cRACE 2nd PCR primer

gar2 RT 2	GATTTCTTTGAAGGTTCGGG	cRACE 2nd PCR primer
gar2 RT 1	CTTCCTTCTTCTTAGATTTC	cRACE 1st PCR primer
gar2 PCR 1	CACTTTTGACTAAGTATACTGGC	cRACE 1st PCR primer
gar2 PCR 2	GAGTTTGGGTTTTTGGG	cRACE 2nd PCR primer
pof9 RT 2	CATAACCTCCAACTTGAACC	cRACE 2nd PCR primer
pof9 RT 1	CAACATTGTTTGGTACCCC	cRACE 1st PCR primer
pof9 PCR 1	CTTTGGAAAGGGACGGC	cRACE 1st PCR primer
pof9 PCR 2	GGTAAGCTAATTTCCGCG	cRACE 2nd PCR primer
Urg1 Real Time	CCCCTGATCATCGTCCC	Northern blot probe primer
Pik1 5' N Blot	GCTGGTAAAAATGTTGTTAC	Northern blot probe primer
Pik1 3' N Blot	TAGTAAATTCCGTTCG	Northern blot probe primer
5' Lsm1 Del	GCGTTTATAAAAGAAATAGAAAGTTAAATCTAAATTAAATTATTAGTTTATGCTACATCCAAATAATAGCTGAAAATCCCACTGGCTATATGT	*lsm1* deletion primer
3' Lsm1 Del	CGAGATAGCTTTCTATCGTGACTGTAAACAAACGGTATAGCAATATTGTGATAAATTTGTTCAACCGTTGTAATTCTAAATGCCTTCTGAC	*lsm1* deletion primer
5' Lsm1 Check	CACTCAAATAAAGTCATC	Checking *lsm1* deletion
3' Lsm1 Check	CATTTAAAAGGGGAATAAAC	Checking *lsm1* deletion
5' Pan3 Del	CCTTAAAGTTGTCGAAAACCTCAATTTCAAGTGCTTCCAATAAAGAGTCCGTACCCCTCACTAGGCCAAAAAATCCCACTGGCTATATGT	*pan3* deletion primer
3' Pan3 Del	CTAAGATTATTTGAACATCGCCGTTCTAACTCCATGAACGCAGTATTAATTGTGGTTTTCACCTAGATAGTTTAATTCTAAATGCCTTCTGAC	*pan3* deletion primer
5' Pan3 Check	CAGTATAAATTCGCCATC	Checking *pan3* deletion
3' Pan3 Check	GTTCGCCTCAAATCGTCAG	Checking *pan3* deletion

Supplementary Table 4. Yeast strains used in this study.

Strain	***Genotype***	***Source***
Wild-type	*h- leu1-32*	Lab stock
ccr4Δ	*h- ccr4::kanMX leu1-32*	This study
cid1Δ	*h- cid1::ura4^{+} leu1-32 ura4-D18*	[8]
cid1Δ (for *urg1* half-life analysis)	*h- cid1::LEU2 leu1-32*	This study
cid1Δccr4Δ	*h? cid1::LEU2 ccr4::kanMX ade6-? leu1-32*	This study
cid1Δpan2Δ	*h? cid1::LEU2 pan2::kanMX ade6-? leu1-32*	This study
cid1ΔparnΔ	*h? cid1::LEU2 parn::hygB^{R} ade6-? leu1-32*	This study
dcp1-ts	*h- dcp1::hygB^{R} leu1::kanr-Padh1-FLAG-dcp1-L69S ura4-D18 ade6*	[21]
lsm1Δ	*h- lsm1::ura4^{+} ade6-M210 leu1-32 ura4-D18*	This study
pan2Δ	*h- pan2::kanMX ade6-? leu1-32*	This study
pan3Δ	*h- pan3::ura4^{+} ade6-? leu1-32 ura4-D18*	This study
parnΔ	*h- parn::hygB^{R} ade6-? leu1-32*	This study
ski2Δ	*h- ski2::ura4^{+} ade6-? leu1-32 ura4-D18*	A. Stevenson
YSP002	*h- ccr4::kanMX ade6-M216 leu1-32 ura4-D18*	[33]
YSP003	*h- pan2::kanMX ade6-M216 leu1-32 ura4-D18*	[33]
YSP004	*h+ parn::hygB^{R} ade6-M216 leu1-32 ura4-D18*	[33]
W303-1A	*MAT*a *leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	N. Proudfoot