Supplemental Data

Polyadenylation Linked to Transcription

Termination Directs the Processing of snoRNA Precursors in Yeast

Pawel Grzechnik and Joanna Kufel

Supplemental experimental procedures

Strain construction and growth

The transformation procedure was as described (Gietz et al., 1992). Strains were generated by one-step PCR procedure (Longtine et al., 1998). To construct strains expressing snR65 from an inducible GAL1 promoter at the SNR65 locus (strains GAL1::SNR65, GAL1::SNR65/rrp6Δ, GAL1::SNR65/trf4Δ and GAL1::SNR65/pap1-5) the region of GAL1 promoter was amplified by PCR using the pFA6a-His3Mx6-pGAL1 plasmid and primers W297 and W298. The C-terminal pap1-2/Trf4-TAP strain was constructed according to (Puig et al., 2001) using W301 and W302 primers. To obtain the double rrp6Δ/trf4-236 mutant RRP6 was disrupted in the FWY9 strain with a His3Mx6 from pFA6a-His3Mx6 plasmid using W307 and W308 primers. TRF4 disruption in the Nrd1-TAP strain was carried out by replacing TRF4 ORF with kanMX6 marker amplified from pFA6a-kanMx6-pGAL1 template (Longtine et al., 1998) using W299 and W300 primers. The trf4-236-HA strain was generated by exchanging the TAP tag and K. lactis TRP1 sequences in the trf4-236::TAP::K.lactis TRP1 gene in the FWY9 strain with a HA- kanMx6 cassette amplified by PCR using the pFA6a-3HA-kanMx6 plasmid and primers W305 and W306. Nrd1-TAP was introduced into trf4-236-HA and GAL1::MTR4 strains using a pBS1539 plasmid (Rigaut et al., 1999) and primers W303 and W304. Strains were grown at 23°C or 30°C either in YPD or YPGal medium (1% yeast extract, 2% Bacto-peptone, 2% glucose or 2% galactose, respectively) or in synthetic complete medium (0.67% yeast nitrogen base, 2% glucose or 2% galactose, supplemented with amino acids and nucleotide bases). Temperature-sensitive strains were grown at 23°C and transferred to 37°C for 2 hours. Transcriptional pulse was induced by addition of 2% galactose to yeast cultures pre-grown in SC medium containing 2% raffinose and 0.08% glucose. Pap1-2 and pap1-5 strains were transferred to 37°C for 30 min prior to the pulse. For pulse-stop experiments 4% glucose was added following the pulse. To deplete
Tfr5 or Mtr4 in *trf4Δ/GAL1::TRF5* or *GAL1::MTR4* and *Nrd1-TAP/GAL1::MTR4* strains, respectively, cells were transferred from YPGal to YPD medium for 12h (*Nrd1-TAP/GAL1::MTR4*) or 20h (*trf4Δ/GAL1::TRF5* and *GAL1::MTR4*), whereas Dis3 in *Tet::DIS3* and *rrp6Δ/Tet::DIS3* strains was depleted by addition of doxycycline for 20h (2.5μg/ml).

**General RNA methods**

Total RNA from yeast cells was isolated using a hot phenol procedure (Schmitt et al., 1990). Northern hybridization and primer extension were essentially as described (Tollervey and Mattaj, 1987). Radioactive probes were either 5'-end γ-32P-labelled oligoprobes or α-32P internally labelled random-primed probe (for SmX2 mRNA) prepared using PCR product as template and DECAprime II Kit (Ambion). 8μg of total RNA or 0.5μg of poly(A)+ RNA and 2μg of total RNA in the case of purified poly(A)+ samples were separated on 6% denaturing polyacrylamide-urea gels, transferred onto nylon membranes and hybridized with oligonucleotide probes listed in supplementary Table S2.

**Western blot analysis**

Western blot analysis was performed using peroxidase-anti-peroxidase antibody to detect Nrd1-TAP and Trf4-TAP and polyclonal anti-Mrf1 antibody followed by horseradish peroxidase-conjugated goat anti-rabbit antibody.

**Calculation of ChIP values**

Nrd1-TAP occupancy at *SNR13* in *trf4Δ, trf4-236* and *GAL1::MTR4* (in nonpermissive conditions, growth in glucose) in Figure 5B was compared to the level in the otherwise wild-type Nrd1-TAP control or *Nrd1-TAP/GAL1::MTR4* cells (in permissive conditions, growth on galactose).

ChIP values for Nrd1-TAP were quantified using the formula 

\[ 2^{-\Delta\Delta Ct} = 2^{-(Ct \text{ IP target gene} - Ct \text{ Input target gene}) - (Ct \text{ IP control} - Ct \text{ Input control})} \]

where "Ct IP" and "Ct Input target gene" are cycle numbers for the *SNR13* gene and "Ct IP" and "Ct Input control" are cycle numbers for non-coding region on chromosome V. ChIP values for Pol II were determined using 

\[ 2^{-\Delta Ct} = 2^{-(Ct \text{ IP} - Ct \text{ background})} \]

where "Ct IP" is cycle number for immunoprecipitate and "Ct background" is cycle number for control without antibodies. ChIP levels for Nrd1-TAP in different strains were corrected for Pol II occupancy.
**Table S1.** Yeast strains used in this work.

| Strain          | Description                                                      | Reference          |
|-----------------|------------------------------------------------------------------|--------------------|
| BY4741          | MATa his3Δ1; leu2Δ0; met15Δ0; ura3Δ0                             | Euroscarf          |
| trf4Δ           | as BY4741 but TRF4::kanMX4                                      | Euroscarf          |
| trf5Δ           | as BY4741 but TRF5::kanMX4                                      | Euroscarf          |
| trf4Δ/GAL1::TRF5| as trf4Δ but HisMX6-pGAL1-HA::TRF5                             | (LaCava et al., 2005) |
| rrp6Δ           | as BY4741 but RRP6::kanMX4                                      | Euroscarf          |
| rrp6Δ/trf4Δ     | as trf4-Δ but RRP6::natMX6                                      | (LaCava et al., 2005) |
| rrp6Δ/trf5Δ     | as trf5-Δ but RRP6::natMX6                                      | (LaCava et al., 2005) |
| W303            | MATa his3-11,15 trp1-1; leu2-3,112 ura3-1 ade2-1                | (Sikorski and Hieter, 1989) |
| CY1243 trf4-836 | as W303 but trf4-ts896::HIS3                                    | (Wang et al., 2000) |
| trf5Δ           | TRF5::LEU2                                                       |                    |
| BMA64           | MATa, ura3-1, ade2-1, his3-11,5, trp1Δ, leu2-3,112, can1-100    | (Baudin et al., 1993) |
| FWY9 trf4-236   | as BMA64 but trf4-236::TAP::K.lactis TRP1                       | (Wyers et al., 2005) |
| FWY10 trf4-236  | as FWY9 but trf5Δ::HIS3                                         | (Wyers et al., 2005) |
| trf5Δ           |                                                                  |                    |
| air1Δ           | as BY4741 but AIR1::kanMX4                                      | Euroscarf          |
| air2Δ           | as BY4741 but AIR2::kanMX4                                      | Euroscarf          |
| air1Δ/air2Δ     | as BY4741 but AIR1::kanMX4                                      | (LaCava et al., 2005) |
| pap1-2          | MATa ade2 his3 trp1 ura3 leu2                                   | (Minvielle-Sebastia et al., 1994) |
| pap1-5          | MATa ade2 his3 trp1 ura3 leu2                                   | (Minvielle-Sebastia et al., 1994) |
| rrp6Δ/pap1-2    | as pap1-2 but RRP6::K.lactis URA3                               | (Milligan et al., 2005) |
| rrp6Δ/pap1-5    | as pap1-5 but RRP6::K.lactis URA3                               | (Milligan et al., 2005) |
pap1-5/trf4Δ as pap1-5 but TRF4::kanMX4 this work
pap1-2/trf4Δ as pap1-2 but TRF4::kanMX4 (Houseley et al., 2007)
Trf4-TAP as BY4741 but TRF4::TAP::HIS3 this work
Trf4-TAP/pap1-2 as pap1-5 but TRF4::TAP::HIS3 this work
GAL1::SNR65 as BY4741 but GAL1::SNR65::HIS3 this work
rrp6Δ/GAL1::SNR65 as rrp6Δ but GAL1::SNR65::HIS3 this work
pap1-5/GAL1::SNR65 as pap1-5 but GAL1::SNR65::HIS3 this work
trf4Δ/GAL1::SNR65 as trf4Δ but GAL1::SNR65::HIS3 this work
YJL1166 MATα ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 (Conrad et al., 2000)
YJL1163 ndr1-102 as YJL1166 but ndr1-102 (Conrad et al., 2000)
ndr1-5 as YJL1166 but ndr1-5 (Conrad et al., 2000)
rrp6Δ/ndr1-102 as ndr1-102 but RRP6::kanMX4 (Houalla et al., 2006)
Nrd1-TAP MATα NRD1::TAP::HIS3MX6 Open Biosystems
Nrd1-TAP/trf4Δ as Nrd1-TAP but TRF4::kanMX4 this work
trf4-236-HA as BMA64 but trf4-236::HA::kanMX4 this work
Nrd1-TAP/trf4-236 as trf4-236-HA but NRD1::TAP::HIS3 this work
rrp6Δ/trf4-236 as FWY9 but RRP6::HISMX6 this work
rrp6Δ/rna14-1 MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 rna14-1 RRP6:: K.lactis TRP1 (Torchet et al., 2002)
rrp6Δ/rna15-1 MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 rna12-2 RRP6:: K.lactis TRP1 (Torchet et al., 2002)
nop1-2 ura3 leu2 nop1-2::HIS3 (Tollervey et al., 1993)
Tet::Dis3 as W303 but LYS2::DIS3 Tet::DIS3 (Dziembowski et al., 2007)
Tet::Dis3 rrp6Δ as Tet::DIS3 but RRP6::kanMX4 (Dziembowski et al., 2007)
GAL1::MTR4 MATα ade2-1 his3 leu2 trp1 ura3 HIS5sp-GAL1-3HA-MTR4 (Torchet et al., 2002)
GAL1::MTR4/Nrd1-TAP as GAL1::MTR4 but NRD1::TAP::K.lactis URA3 this work
**Table S2. Oligonucleotides used in this work.**

| Primer | Primer name | Sequence          |
|--------|-------------|-------------------|
| hybridization probes | primer name | sequence          |
| W035   | snR13       | CAACTCGAGCCTAATGCACTC |
| W036   | snR33       | CTTTCAATCTCTGCTCTCC |
| W037   | snR3        | CAACTAGCAATCCACTCGAG |
| W038   | snR43       | TTCAAAGCTTGATCTTCTCC |
| W270   | snR46       | TTAGGCGCTCGTTTGAATCC |
| 261    | U6          | AAAACGAAATAAATTTCTTGTAAAAC |
| 205    | U18         | GTCAGATACTGTGATAGTC |
| 202    | U14         | TCACTCAGACATCCTAGG |
| W076   | snR68-2     | AAGAGTCAATTTTCCTCGTA |
| W085   | snR64-2     | GATGTTTCTCGTCACTTGG |
| W271   | snR65       | GCTTTTCAGATACTATCTAGC |
| W045   | snR50       | CTGCTGCAAATTGCTACCTC |
| W309   | 5smx3       | AGCGAGAGCGATGATATCAG |
| W310   | 3smx3       | TTAGTTCGGCAGCTCCCTG |
| primer extension | primer name | sequence          |
| 262    | U1          | CAATGACTTCAATGAACAAATTAT |
| W288   | 3snr65sp    | ATAACTCAAATCAGCTCATAAC |
| W289   | 65RTligP    | TACCAAGAGTTACAAAATC |
| W041   | Trs31son    | GTTGAAATTATTTGTGAGAC |
| RT-PCR | Primer name | Sequence          |
| W290   | ADAPT-dT    | CACTCGAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT |
| W291   | 5snR65      | TAAAATGATGATTTTTTTAAAC |
| W292   | 5snR13      | AGGAAGTTTTTTCCTTTTTATATG |
| CR-RT-PCR | Primer name | Sequence          |
| W091   | 68RTlig     | GATAACGCAGTAAATAAATG |
| W092   | 68PCRlig    | GTACAGTCTCTTTTATAATC |
| W272   | 50RTlig     | AATCTGCTGCAAATTGTAC |
| W273   | 50PCRlig    | TGAATCAAACAAAGATTAAC |
| W274   | 13Hlig      | AAAAAGGA AAAAACCTTCT |
| W275   | 13RTlig     | ACAGCAACTCGAGCCAAATG |
| W276  | 13PCRlig | TTGCCAAATCAGTAACGGTG |
| W277  | 3Hlig    | ATTAGTACTTTTAGGACAAAG |
| W278  | 3RTlig   | CGCTTATCACGAATAAGACC |
| W279  | 3PCRlig  | CATTTATAAGAACTCGAGTG |
| W280  | 43RTlig  | TATAGAACCCATGTCCCGTG |
| W281  | 43PCRlig | TTGATACAAACGGTAGACGGC |
| W282  | 65RTlig  | GTTAAGAAGATTTCAAGATTC |
| W283  | 65PCRlig | AGCTGATTGTGATTATGGGCG |
| W284  | 65RTligD | GCTTTCAGATACTATCTAG |
| W285  | 65PCligD | TTATGATTACAGTGTTTTTC |
| W286  | U18ligRT | TTCCCATCATAAACACGGAC |
| W287  | U18ligPC | GAGATGTTGTTGACTATCAG |

### ChIP

| W293  | 13-2up   | CTGACCTTTTAACTTTCCCGTAG |
| W294  | 13-2low  | CTGTGCTTCCGTGTCTTTGCTCTG |
| W295  | 5ChV     | CTGTCAAAATATGGGGCCGTAG |
| W296  | 3ChV     | CCATACCCCTCGGTTACAAAC |
| W304  | 5sn65    | GCTTCACCAGATGTTCTTGTC |
| W304  | 3sn65    | TACCAAGAGTTACAAAAATCG |

### strain construction

| W297  | 5GLSNR65 | TTTTATGCGCGCCTCCTCTTCCTTTAAAAAAAAAAATTTACATATATAAC |
| W298  | 3GLSNR65 | GTGGGGGGGGGAGTGAAGTACACGCCCTTCCCTTCCTTCAGGTTCTCAG |
| W299  | 5TRF4d   | ATGGGGGGAAGTGAAGTACACGCCCTTCCCTTCCTTCAGGTTCTCAG |
| W300  | 3TRF4d   | ATGGGGGGAAGTGAAGTACACGCCCTTCCCTTCCTTCAGGTTCTCAG |
| W301  | 5Trf4TAP | ATGGGGGGAAGTGAAGTACACGCCCTTCCCTTCCTTCAGGTTCTCAG |
| W302  | 3Trf4TAP | ATGGGGGGAAGTGAAGTACACGCCCTTCCCTTCCTTCAGGTTCTCAG |
| W303  | 5TAPnrd1 | ATGGGGGGAAGTGAAGTACACGCCCTTCCCTTCCTTCAGGTTCTCAG |
CAACAAAGCTCCATGGAAAAGAGAAG

W304 3TAPnrd1  GAACATAGGAAAAAACAGAAATTATATATAGAGGTAGATT
AGTTTTATGTTACGACTCACTATAGGG

W305 5HAtrf4fa  CTGTCTCTAGCGAAGATGATGATGAAGATGGATATAATCCT
TATACCCCTCGGATCCCCGGGTAAATTAA

W306 3TAPmycfa  CATGATTGCATGGTATCACTACACACATCCCATATACCCCG
GTATCTCTCGAATTCGAGCTCGTTTAAAC

W307 5RRP6d  TAGACGAAATAGGAACAACAAACAGCTTATAAGCACCCAA
TAAGTGGCTTCGGGATCCCCGGGTAAATTAA

W308 3RRP6d  ATGAAAAATTACCATAATTTTATAAATAAAAAATACGCTTGT
TTTACATAAGAATTCGAGCTCGTTAAAC

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Figure S1.
Box C/D and box H/ACA snoRNA precursors are polyadenylated in the *rrp6Δ* strain.
Northern hybridization of total and poly(A)+ RNA (lane A+) from the *rrp6Δ* strain probed for box C/D (snR64, snR68, U14) and box H/ACA (snR46 and snR33) snoRNAs. U6 (lane 1) and *SMX2* mRNA (lane 2) are used as loading controls for total and poly(A)+ RNAs, respectively.

Figure S2
Both I-pA and II-pA precursors are detected during the transcriptional pulse in wild-type cells.
RT-PCR analysis of polyadenylated pre-snR65 in *GAL1::SNR65* strain where transcription was induced by addition of galactose for times indicated. Reverse transcription was performed using using ADAPT-oligo(dT)30 and cDNA was amplified using ADAPT-oligo(dT)30 and a primer specific for mature snR65. The asterisk indicates primer-dimers.

Figure S3
Transcriptional pulse of snR65 under the control of the *GAL1* promoter generates mature (M) snoRNA in wild-type, *trf4Δ, pap1-2* strains but only untrimmed semi-mature (M*) species in *rrp6Δ* cells. Cells were grown at 23°C in SC medium (2% raffinose, 0.08% glucose) and transcription of snR65 was induced for times indicated by addition of galactose. I-pA and II-pA, polyadenylated precursors from respective termination sites; I*, oligoadenylated precursor from site I; M*, semi-mature species; M, mature snoRNA.

Figure S4
Deadenylases are not involved in removal of poly(A) tails.
Northern analysis of snR65 in *ccr4Δ/caf1Δ* and *ccr4Δ/pan2Δ* strains. Total RNA (lower panel with mature snoRNA) and the poly(A)+ fraction (upper panel). U6 and SmX2 mRNA, loading controls for total and poly(A)+ RNAs, respectively.

Figure S5
TRAMP components Air1/2 are also involved in snoRNA processing. Northern analysis of polyadenylated snR65 in *trf4Δ, air1Δ, air2Δ, air1Δ/air2Δ* and *trf4-ts836/trf5Δ* strains. Only deletion of both Air proteins result in the phenotype comparable to that in the *trf4Δ* strain as
had been observed for several effects characteristic for TRAMP mutants (LaCava et al., 2005; Wyers et al., 2005). U6 is used as a control.

**Figure S6**
Mutation in Nrd1 and lack of Trf4 shift polyadenylated snR65 precursors towards site II. RT-PCR analysis of polyadenylated pre-snR65 in wild-type, *nrd1-102* and *trf4Δ* strains grown at 23°C or shifted to 37°C for 2 hours. Reverse transcription was performed using using ADAPT-oligo(dT)$_{30}$ and the cDNA was amplified using ADAPT-oligo(dT)$_{30}$ and a primer specific for mature snR65.

**Figure S7**
Transcription rate of endogenous snR13 is not altered in the absence of Trf4 and by the *trf4-236* mutation (A) or following depletion of Mtr4 (B). SnR65 under the control of *GAL1* promoter is transcribed with similar rates in wild-type and *trf4Δ* strains (C).

Pol II occupancy along *SNR13* or *SNR65* was analysed by ChIP using 8WG16 antibodies against the CTD of Pol II in *Nrd1-TAP* (wt), *Nrd1-TAP/trf4Δ* (*trf4Δ*), *Nrd1-TAP/trf4-236* (*trf4-236*) strains (A), in *Nrd1-TAP/GAL1::MTR4* (*GAL1::MTR4*) cells before (GAL) and after (GLU, 12 hours) depletion (B) and in *GAL1::SNR65, trf4Δ/GAL1::SNR65* cells grown in GAL (C). Error bars reflect standard deviation of three experiments.

**Figure S8**
Deletion of Trf4 and depletion of Mtr4 do not affect the level of Nrd1 protein. Western blot of Nrd1-TAP, detected with peroxidase-anti-peroxidase antibodies, in *Nrd1-TAP, Nrd1-TAP/trf4Δ* and *Nrd1-TAP/GAL1::MTR4* strains. Mrf1 protein detected with protein-specific antibodies was used as a loading control.