

SUPPLEMENTARY DATA

Supplementary methods: Pol I minigene construction

The promoter-containing fragment was produced by PCR on genomic DNA with the oligos “NotI-prom-fw” and “XhoI-prom-rev”, the human β-globin fragment from a plasmid containing the human β-globin gene with the oligos “HBfragm-XhoI-fw” and “HBfragm-Sall-rev”, the terminator fragment from genomic DNA with the oligos “XhoI-term-fw” and “KpnI-term-rev”. β-globin and terminator fragments were first digested with XhoI+Sall and XhoI+KpnI, respectively, then ligated between them, amplified by PCR and cloned into pBluescript SK(+) at the XhoI and KpnI sites. The promoter fragment was inserted in the vector pRS426 (NotI/XhoI). The β-globin + terminator fragment was then subcloned from pBluescript into this construct (XhoI/KpnI) to obtain the full WT minigene. All the terminator mutants were first prepared in pBluescript SK(+) then subcloned in the promoter-containing pRS426. Deletion of the Rnt1 cleavage site was obtained by PCR and re-ligation of the product using the oligos “TF-noRnt1-fw” and “TF-noRnt1-rev”, deletion of T1 with the oligos “TF-noT1-fw” and “TF-noT1-rev”. Ribozyme and mutant ribozyme were obtained by PCR on a plasmid template with the oligos “RZ-fw” and “RZ-rev” then cloned in place of Rnt1 cleavage site and T1 site respectively. Point Mutagenesis of the Reb1 binding site was performed using the mutagenic primers “MutReb1BS” and “MutReb1BS-anti”. The double mutant ΔT1/mutReb1BS was obtained from the mutReb1BS construct by deletion of T1 with the oligos “TF-noT1mut-fw” and “TF-noT1-rev”. Deletion of the termination fragment was obtained from the WT construct in pRS426 by PCR and re-ligation with the oligos “noTF-fw” and “noTF-rev”. The sequence of the oligos employed is reported in Supplementary Table 2.

Supplementary Table S1: Yeast strains

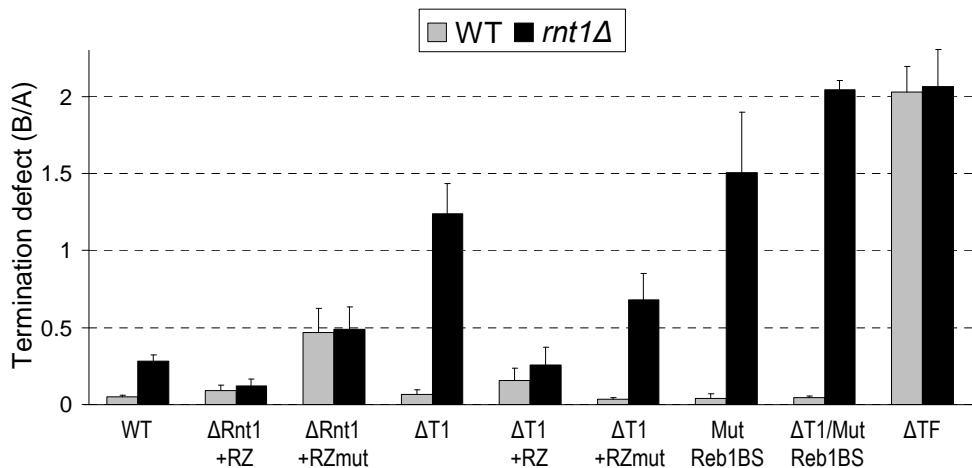
Strain	Genotype
BY4742 (<i>RPA12</i>)	Mat-a <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>
<i>rpa12Δ</i>	Mat-a <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rpa12::kanMX6</i>
BMA64 (<i>RNT1</i>)	Mat-a <i>ura3-1 trp1 ade2-1 leu2-3,112 his3-11,15</i>
<i>rnt1Δ</i>	Mat-a <i>ura3-1 trp1 ade2-1 leu2-3,112 his3-11,15 rnt1::TRP1</i>
J342 (<i>pGAL-REB1</i>)	Mat-a <i>ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 reb1Δ::LEU2 pBM272-41 (P_{GAL} REB1, HIS3)</i>
FD4D (<i>RAT1 SEN1</i>)	Mat-a <i>leu2 trp1Δ63 ura3</i>
<i>rat1-1 sen1-1</i>	Mat-a <i>leu2 trp1Δ63 ura3 rat1-1 sen1-1</i>

Supplementary Table S2: Primer sequences

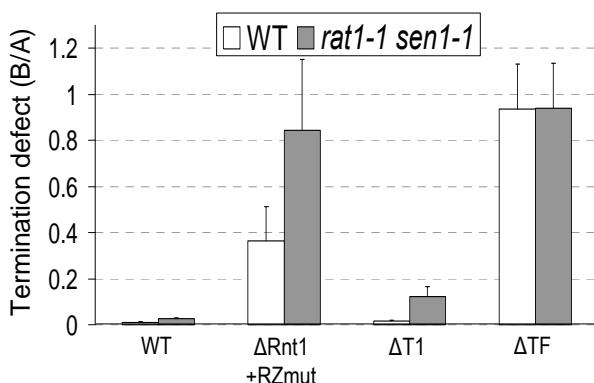
Name	Sequence (5`-3`)
RNaseH	CCAGTACCCACTTAGAAAGA
R-fw	AAGTAGACTGAACAAGTCTCTA
R-rev	TCTCTAAACTAGGCCCCGGC
Ext-rev	CATCAGGGCTGTTGCCAATG
NotI-prom-fw	GGTTACGC GGCGCAACGGTGCACTTGGCGGAAAG
XhoI-prom-rev	GTCAGTCTCGAGTCGAACTTGTCTCACTGCTTCG
HBfragm-XhoI-fw	CGAGTACTCGAGGCAATGATGTATTAAATTATTCTG
HBfragm-SalI-rev	ATGGTTGTCGACGAATCCTTTCTGAGGGATGAATAAG
XhoI-term-fw	GATGATCTCGAGGTTTTATTCTTAAGTGGGTAC
KpnI-term-rev	CTAGAAGGTACCCAATACATGTTTTACCCGGATC
TF-noRnt1-fw	GAGAGAAGTAGACTGAACAAGTCTC
TF-noRnt1-rev	CTCGACGAATCCTTTCTGAGG
TF-noT1-fw	GAATTCTATGATCCGGGTAAAAAC
TF-noT1mut-fw	GAATTCTATGATCCTTGATGTTACATG
TF-noT1-rev	GAGACTTGGTCAGTCTACTTCTC
RZ-fw	CCTGTCACCGGATGTGTTTC
RZ-rev	CCTGTTCGTCCTCACGGAC
MutReb1BS	TTTATTGTCTTAAGAATTCTATGATCCTGTAAAAACA TGTATTGGGTACCCAATTC
MutReb1BS-anti	GAATTGGGTACCCAATACATGTTTTACAAGGATCATA GAATTCTTAAGACAAATAAA
noTF-fw	GGTACCCAATTGCCCTATAG
noTF-rev	CTCGACGAATCCTTTCTGAGG
A-fw	CTTAAACTCCATGAAAGAAGG
A-rev	CTCGACGAATCCTTTCTGAGG
B-fw	CAACTTAATGCCCTGCAGCAC
B-rev	CATTCAAGGCTGCGCAACTGTTG
3C-1	GGCACCTGTCACTTGGAA
3C-2	GTCATGGAGTACAAGTGTGAGG
3C-3	GAAAGCAGTTGAAGACAAGTCG
3C-4	GGGAATGTGGGAGGTCAGTGC
4A-1	GAAGAGCTAGTCAAACCTTGG
4A-2	GAATTGTTCAAGTCTACTTCTC
4A-3	GAGAAGTAGACTGAACAAGTC
4A-4	CTGCGCAACTGTTGGGAAGGGC

Supplementary Figure S1: Raw data for the graphs normalized towards Δ TF in Figure 4B, 4C and 4D

Termination efficiency



Rat1/Sen1 effect



Reb1p depletion

