Termination of non-coding transcription in yeast relies on both a CTD-interaction domain and a CTD-mimic in Sen1

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List of supplementary material:

-Supplementary figure 1.

-Tables S1, S2, S8, S9, S10 and S11.

-Supplementary references.

Material provided as separate files:

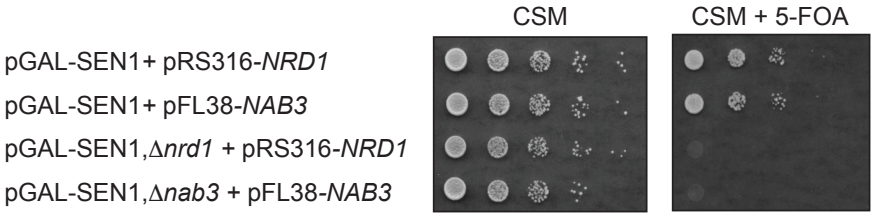
-Table S3: quantification of termination defects at snoRNAs in *sen1ΔNter* and *sen1ΔNterΔNIM* mutants.

-Table S4: protein-coding genes upregulated in *senΔNter* relative to the wt.

-Table S5: protein-coding genes upregulated in *sen1ΔNterΔNIM* relative to the wt.

-Table S6: protein-coding genes downregulated in *senΔNter* relative to the wt.

-Table S7: protein-coding genes downregulated in *sen1ΔNterΔNIM* relative to the wt.



Supplementary figure 1: Overexpression of Sen1 does not bypass the requirement of Nrd1 and Nab3 for cell viability. Cells expressing *SEN1* from pGAL in a wt context or combined with deletions of *NRD1* or *NAB3* covered by *URA3*-centromeric plasmids harbouring the respective *NRD1* or *NAB3* alleles were grown in CSM in the presence of galactose to induce overexpression of *SEN1*. Then, cells were plated on CSM containing galactose in the absence or in the presence of 5-FOA to monitor the capacity of cells to lose the *URA3* plasmids. The absence of growth of *Δnrd1* and *Δnab3* cells on 5-FOA plates implies that these genes are still essential even when *SEN1* is overexpressed.

Table S1: Equilibrium binding of Nrd1 CID to Sen1 and Trf4 NIMs monitored by fluorescence anisotropy.

	Sen1 NIM	Trf4 NIM
	Kd (μM)	Kd (μM)
wt	1.2 ± 0.02	0.9 ± 0.02
L20D	16.9 ± 1.5	13.4 ± 1.2
K21D	8.0 ± 0.8	6.1 ± 0.4
S25D	76.1 ± 14.2	53.9 ± 8.2
R28D	78.2 ± 11.5	63.4 ± 4.0
I130A	7.6 ± 0.3	4.5 ± 0.2
I130K	36.7 ± 4.6	14.1 ± 1.2
I130N	8.9 ± 0.5	6.1 ± 0.3
M126A	1.4 ± 0.07	1.1 ± 0.02
R133A	1.8 ± 0.08	1.4 ± 0.02
R133D	2.0 ± 0.1	1.2 ± 0.05
R133G	1.6 ± 0.04	1.3 ± 0.04

Table S2: NMR and refinement statistics for the Nrd1 CID–Sen1 NIM complex

	Nrd1 CID-Sen1 NIM complex
NMR distance & dihedral constraints	
Distance restraints	
Total NOEs	2499
Intra-residue	654
Inter-residue	1845
Short	1273
Medium	645
Long	581
Hydrogen bonds	100
Intermolecular distance restraints	65
Total dihedral angle restraints[a]	222
Structure statistics[b]	
Violations (mean and s.d.)	
Number of distance restraint violations > 0.5 Å	0.05 ± 0.22
Number of dihedral angle restraint violations > 15º	1.1 ± 1.2
Max. dihedral angle restraint violation (º)	29.03 ± 27.63
Max. distance constraint violation (Å)	0.027 ± 0.12
Deviations from idealized geometry[b]	
Bond lengths (Å)	0.0037 ± 0.00008
Bond angles (º)	1.673 ± 0.013
Average pairwise r.m.s.d (Å)[b]	
Nrd1 CID (6-82,91-135,143-147,149-153)	
Heavy atoms	1.1
Backbone atoms	0.7
Sen1 NIM (2052-2063)	
Heavy atoms	1.9
Backbone atoms	1.3
Complex	
All complex heavy atoms	2.5
All complex backbone atoms	2.0
Ramachandran plot statistics[c]	
Residues in most favoured regions (%)	96.7
Residues in allowed regions (%)	3.2
Residues in generously allowed regions (%)	0.1
Residues in disallowed regions (%)	0.0

[a] α-helical dihedral angle restraints imposed for the backbone based on the CSI.
[b] Calculated for an ensemble of the 20 lowest energy structures.
[c] Based on Procheck analysis [2].

Table S8: Antibodies used in this work.

Name	Reference/provider	Working dilution	Use
Anti-HA F7	Sc-7392/ Santa Cruz	1:1000	Sen1-HA detection in figures 1A, 1C-D, 6A
Anti-HA 12CA5	11 666 606 001/ Sigma	2.5 ug/reaction	Sen1 coIP in figure 1D
Anti-CBP, calmodulin binding protein	07-482/ Millipore	1:1000	Nrd-CBP detection in figure 1A, Sen1-CBP detection in figure 6B
Anti-Air2	S. Vanacova	1:1000	Air2 detection in figure 1A
Anti-Rpb1 y80	Santa Cruz (not available any more)	1:1000	Rpb1 detection in figure 6A, 6B
Anti-phospho-CTD Ser-5 clone 3E8	04-1572/ Millipore	1:1000	Detection of S5P-CTD of RNAP II in figure 6B
Anti-phospho-CTD Ser-2 clone 3E10	04-1571/ Millipore	1:1000	Detection of S2P-CTD of RNAP II in figure 6B
PAP, peroxidase anti-peroxidase	P1291-1ML/ Sigma	1:3000	Used for Protein A detection on TAP tag.
Anti-Nrd1	Covalab	1:3000	Nrd1 detection in figures 1 and 6
Anti-Nab3	Covalab	1:3000	Nab3 detection in figures 1 and 6
Anti-His	H1029,Sigma	1:2000	His_6-GST-Sen1 Cter and His_6-GST in figure 6E.

Table S9: Yeast strains used in this work.

Number	Name	Genotype	Source
DLY17	W303	*ura3-1, ade2-1, his3-11,5, trp1-1, leu2-3,112, can1-100*	(Thomas and Rothstein, 1989)
DLY671	BMA	*as W303, ∆trp1*	F. Lacroute
DLY814	*Δrrp6*	*as W303, rrp6::KAN*	(Porrua et al., 2012)
DLY1657	*sen1-HA*	*as BMA, Sen1::HA::KAN*	Tudek et al, 2014
DLY2014	*nrd1 ΔCID-TAP, sen1-HA*	*as BMA, nrd1ΔCID (Δ6-150)::TAP::HIS3*	Tudek et al, 2014
DLY2769	*sen1ΔNIM*	*as BMA, sen1ΔNIM (Δ2052-2063)*	This work
DLY2982	*sen1ΔNIM-HA*	*as BMA, sen1ΔNIM (Δ2052-2063)::HA::KAN*	This work
DLY1737	*nrd1-TAP, sen1-HA*	*as BMA, NRD1::TAP::HIS5 Sen1::HA::KAN*	Tudek et al, 2014
DLY2622	*nrd1-TAP, sen1ΔNIM-HA*	*as BMA, NRD1::TAP::HIS5, sen1ΔNIM (∆2052-2063)::HA::KAN*	This work
DLY2657	*sen1ΔCter-HA*	*as BMA, sen1ΔCter (∆1930-2231)::HA::KAN*	This work
DLY2658	*nrd1-TAP, sen1ΔCter-HA*	*as BMA, NRD1::TAP::HIS5, sen1ΔCter (∆1930-2231)::HA::KAN*	This work
DLY2695	*sen1ΔCter-HA, Δrrp6*	*as BMA, sen1ΔCter (∆1930-2231)::HA::KAN, rrp6::URA*	This work
DLY2694	*sen1-HA, Δrrp6*	*as BMA, sen1::HA::KAN, rrp6::URA*	This work
DLY2770	*sen1ΔNIM, Δrrp6*	*as BMA, sen1ΔNIM, rrp6::KAN*	This work
DLY2767	*Δsen1/*pFL38-*SEN1*	*as BMA, sen1::KAN, harbouring plasmid pFL38-SEN1*	This work
DLY1656	P_{GAL1}- *TAP-SEN1*	*as BMA, TRP1::Pgal::TAP::SEN1*	(Porrua et al., 2012)
DLY2778	P_{GAL1}- *TAP-sen1ΔNIM*	*as BMA, TRP1::Pgal::TAP::sen1ΔNIM (Δ2052-2063)*	This work
DLY2692	P_{GAL1}- *TAP-sen1ΔNter*	*as BMA, TRP1::Pgal::TAP::sen1∆Nter (∆1-975)*	This work
DLY2779	P_{GAL1}- *TAP-sen1ΔNter ΔNIM*	*as BMA, TRP1::Pgal::TAP::sen1∆Nter (∆1-975) ΔNIM (Δ2052-2063)*	This work
DLY2060	P_{GAL1}- *TAP-SEN1, Δrrp6*	*as BMA, TRP1::Pgal::TAP::SEN1, rrp6::KAN*	(Porrua et al., 2012)
DLY2780	P_{GAL1}- *TAP-sen1ΔNIM, Δrrp6*	*as BMA, TRP1::Pgal::TAP::sen1ΔNIM (Δ2052-2063), rrp6::KAN*	This work
DLY2698	P_{GAL1}- *TAP-sen1ΔNter,Δrrp6*	*as BMA, TRP1::Pgal::TAP::sen1∆Nter (∆1-975), rrp6::KAN*	This work
DLY2781	P_{GAL1}- *TAP-sen1ΔNterΔNIM, Δrrp6*	*as BMA, TRP1::Pgal::TAP::sen1∆Nter (∆1-975) ΔNIM (Δ2052-2063), rrp6::KAN*	This work
DLY2782	*sen1-AID*	*as BMA,SEN1-AID::KAN::OsTIR1*	This work

DLY2788	*sen1-AID, Δrrp6*	*as BMA,SEN1-AID::KAN::OsTIR1, rrp6::URA*	This work
DLY2724	*TAP-sen1/*pFL38-*SEN1*	*as BMA, TAP::SEN1, harbouring plasmid pFL38-SEN1*	This work
DLY2725	*TAP- sen1ΔNter /*pFL38-*SEN1*	*TAP::sen1∆Nter (∆1-975), harbouring plasmid pFL38-SEN1*	This work
DLY2726	*TAP- sen1ΔNterΔNIM /*pFL38-*SEN1*	*as BMA, TAP::sen1∆Nter (∆1-975) ΔNIM (Δ2052-2063), harbouring plasmid pFL38-SEN1*	This work
DLY3152	P_{GAL1}- *TAP-SEN1, Δnrd1/ pRS316-NRD1*	*as BMA, TRP1::Pgal::TAP::SEN1, nrd1::KAN; harbouring plasmid pRS316-NRD1*	This work
DLY3187	P_{GAL1}- *TAP-SEN1, Δnab3/ pFL38-Nab3*	*as BMA, TRP1::Pgal::TAP::SEN1, nab3::KAN; harbouring plasmid pFL38-NAB3*	This work
DLY3081	*rpb3-FLAG*	*as BMA, RPB3::3xFLAG::natMX6*	This work
DLY3151	*rpb3-FLAG, sen1-AID*	*as BMA, SEN1-AID:KAN::OsTIR1, RPB3::3xFLAG::natMX6*	This work

Table S10: plasmids used in this work.

Name	**Description**	Source
pBS1761	Ap^r, *ori*ColE1; plasmid bearing cassette for N-terminal tagging with PGAL1-TAP	(Finoux and Séraphin, 2006)
pDL708 (pU6H3HA)	Ap^r, *ori*ColE1; plasmid bearing cassette for C-terminal tagging with *HA*	(Finoux and Séraphin, 2006)
pDL772	Ap^r; *ori*ColE1; derivative of pFL38 (URA) bearing yeast *SEN1*	F. Lacroute
pDL693	Ap^r; *ori*ColE1; derivative of pFL39 bearing yeast *SEN1*	F. Lacroute
pDL703	Ap^r; *ori*ColE1; derivative of pFL39 bearing *sen1ΔNIM*	This work
pETM30	Kan^r; oriColE1; vector for overexpression of proteins from the T7 promoter	R. Stefl
pDL834	Ap^r; *ori*ColE1; derivative of pFL39 bearing *sen1ΔNter*	This work
pDL835	Ap^r; *ori*ColE1; derivative of pFL39 bearing *sen1ΔNterΔNIM*	This work
pDL846	Kan^r; oriColE1; derivative of pETM30 carrying the C-terminal domain of Sen1 (aa 1931-2231) under the control of the T7 promoter	This work
pDL848	Kan^r; oriColE1; derivative of pETM30 carrying the ΔNIM version of the C-terminal domain of Sen1 (aa 1931-2231) under the control of the T7 promoter	This work
pDL856	Ap^r; *ori*ColE1; derivative of pFL39 bearing yeast se*n1-HA*	This work
pDL858	Ap^r; *ori*ColE1; derivative of pFL39 bearing *sen1ΔNIM-HA*	This work
pDL857	Ap^r; *ori*ColE1; derivative of pFL39 bearing *sen1ΔNter-HA*	This work
pDL859	Ap^r; *ori*ColE1; derivative of pFL39 bearing *sen1ΔNterΔNIM-HA*	This work
pDL876	Ap^r; *ori*ColE1; derivative of pFL39 bearing the chimeric *nrd1CID* (aa 1-153) - *sen1ΔNter*	This work
pDL877	Ap^r; *ori*ColE1; derivative of pFL39 bearing the chimeric *nrd1CID* (aa 1-153) - *sen1ΔNterΔNIM*	This work
pRS_NC	Ap^r; oriColE1; plasmid bearing CID-His6 under the control of the T7 promoter	(Kubicek et al., 2012)
pRS_NC_L20D	Kan^r; oriColE1; plasmid bearing CID(L20D)-His6 under the control of the T7 promoter	(Vasiljeva et al., 2008)
pRS_NC_K21D	Kan^r; oriColE1; plasmid bearing CID(K21D)-His6 under the control of the T7 promoter	(Vasiljeva et al., 2008)
pRS_NC_S25R	Ap^r; oriColE1; plasmid bearing CID(S25R)-His6 under the control of the T7 promoter	(Kubicek et al., 2012)
pRS_NC_R28D	Ap^r; oriColE1; plasmid bearing CID(R28D)-His6 under the control of the T7 promoter	(Kubicek et al., 2012)
pRS_NC_I130A	Ap^r; oriColE1; plasmid bearing CID(I130A)-His6 under the control of the T7 promoter	(Tudek et al., 2014)
pRS_NC_I130K	Ap^r; oriColE1; plasmid bearing CID(I130K)-His6 under the control of the T7 promoter	(Tudek et al., 2014)
pRS_NC_I130N	Ap^r; oriColE1; plasmid bearing CID(I130N)-His6 under the control of the T7 promoter	(Tudek et al., 2014)
pRS_NC_R133A	Ap^r; oriColE1; plasmid bearing CID(R133A)-His6 under the control of the T7 promoter	(Tudek et al., 2014)
pRS_NC_R133G	Ap^r; oriColE1; plasmid bearing CID(R133G)-His6 under the control of the T7 promoter	This work
pRS_NC_R133D	Ap^r; oriColE1; plasmid bearing CID(R133D)-His6 under the control of the T7 promoter	This work

Table S11: List of oligonucleotides used in this work.

Name	Sequence (5'-3')	Information/use
DL1119	AAGTGACGAAGTTCATGCTA	Forward oligo to generate by PCR a probe to detect snR13 read-through region.
DL1367	GGCCCAACAGTATATTCATATCC	Reverse oligo to generate by PCR a probe to detect snR13 read-through region.
DL474	GCAAAGATCTGTATGAAAGG	Forward oligo to generate by PCR a probe to detect NEL025C.
DL480	ATCTGACCAGGTCAAGCTAC	Reverse oligo to generate by PCR a probe to detect NEL025C.
DL2505	GTGTGTGGACAATCGATTTGC	Forward oligo to generate by PCR a probe to detect snR33 3'precursor and read-through region
DL2506	GCATTGGCTCGATTGTCAAC	Reverse oligo to generate by PCR a probe to detect snR33 3'precursor and read-through region
DL4178	GCGTGATACTGACCGATACC	Forward oligo to generate by PCR a probe to detect snR82 3'precursor and read-through region
DL4179	TTGCCTTGCTATTATGTGTCC	Reverse oligo to generate by PCR a probe to detect snR82 3'precursor and read-through region
DL4278	GCTTCCATACGAACGGCTCA	Forward oligo to generate by PCR a probe to detect YDR379C-A and CUT526 read-through.
DL4279	AGGGTGTGAAAAGGTGGCAA	Reverse oligo to generate by PCR a probe to detect YDR379C-A and CUT526 read-through.
DL1154	CCTATAACAACAACAACATG	Forward oligo to generate by PCR a probe to detect snR47 RNA.
DL1157	ATAGCCATTAGTAAGTACGC	Reverse oligo to generate by PCR a probe to detect snR47 RNA.
DL2627	ATTCAAAAGCGAACACCGAATTGAC CATGAGGAGACGGTCTGGTTTAT	Reverse oligo used as oligo probe to detect U4 snRNA

Supplementary references:

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