

**Appendix: Antisense transcription-dependent chromatin signature modulates sense transcript dynamics**

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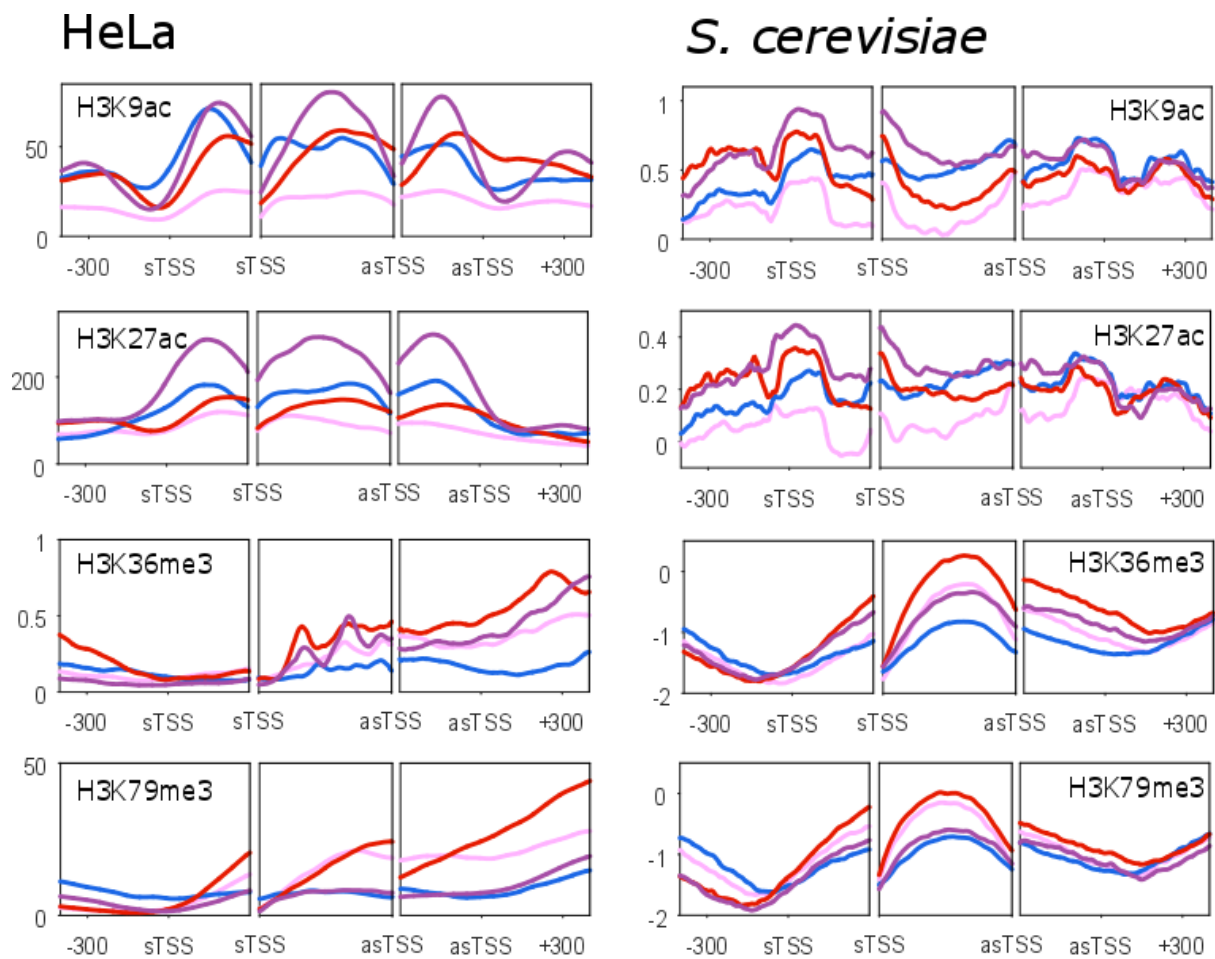
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low sense, low antisense (n = 192)  
 low sense, high antisense (n = 149)  
 high sense, low antisense (n = 140)  
 high sense, high antisense (n = 198)

### Appendix Figure S1

Levels of histone acetylation and trimethylation in gene classes with varying levels of antisense transcription, in both HeLa and *S. cerevisiae*. Shown are average levels in four different gene classes as shown at bottom of figure. In all cases, *low* was any level of sense/antisense transcription (measured by NET-seq) beneath the 30<sup>th</sup> percentile for all genes, and *high* was any level above the 70<sup>th</sup> percentile. For each modification in each species, the three panels are the same format as shown in fig 2B.

## Supplementary Tables

**Appendix Table S1. Primer sequences used for qPCR.**

Primer Name	Sequence (5'-3')
<i>GAL1</i> -2 F	CCGGTCGCGTTCCTGAAACG
<i>GAL1</i> -2 R	GCCAGGTTACTGCCAATTTT
<i>GAL1</i> -1 F	TGGAAAAGCTGCATAACCACT
<i>GAL1</i> -1 R	GCGCTAGAATTGAACTCAGGT
<i>GAL1</i> 1 F	CGCTTATGATGCTAAACCGG
<i>GAL1</i> 1 R	CGCAAAGCATATCAAATCA
<i>GAL1</i> 2 F	CCGTCAAAGTTTTGAACGAGA
<i>GAL1</i> 2 R	CAGAAGGATCAATTGTGACATAAG

**Appendix Table S2. Primer sequences used to generate the strand-specific Northern blotting probes**

Primer Name	Sequence (5'-3')
<i>GAL1</i> F	TGTGTCGGACTGGTCTAATT
<i>GAL1</i> R	GATCCATACCGCCATTGTTA

**Appendix Table S3. Sequences of the *GAL1* sense RNA-FISH probes**

Underlined nucleotides were ordered as amino-allyl-dTs for subsequent labelling with Cy3 fluorescent dye.

Probe Name	Sequence (5'-3')
<i>GAL1</i> Sense probe 1	<u>T</u> <u>I</u> ATGCTCGGGCAC <u>I</u> TTTCGGCCAAT <u>I</u> GG TCTTGGTAA <u>I</u> TCCTTTGCGCT
<i>GAL1</i> Sense probe 2	AGGA <u>I</u> CAATTGTGACATAAGAACCG <u>I</u> CC AACGGCAA <u>I</u> CGAACTTCC <u>I</u> T
<i>GAL1</i> Sense probe 3	<u>I</u> ACATCACCC <u>I</u> CACAGAAGACT <u>I</u> GCAGC CCGGCCAGAGGAGCAC <u>I</u> GGCAA
<i>GAL1</i> Sense probe 4	<u>I</u> CCAGGGCCCATAT <u>I</u> TCGCTTTAACAACA GCTAAAGCAACGGCACAA <u>I</u> TG
S65T pFA6a-GFP probe 1	CCTCTTCTTGAAAAGTGACC
S65T pFA6a-GFP probe 2	ATCTACCACTACAATTACCC

S65T pFA6a-GFP probe 3	TAAAAGACAGTCACCTCTCC
S65T pFA6a-GFP probe 4	CGTTGTATGCCTTTTGAATG
S65T pFA6a-GFP probe 5	AACGTGATGACCTTTTGATG
S65T pFA6a-GFP probe 6	CAAGGTACCGGTTGTGAACA
S65T pFA6a-GFP probe 7	AGTGAATACCACAAGTTACG
S65T pFA6a-GFP probe 8	CTAGTATACTTTGCCGTA
S65T pFA6a-GFP probe 9	AAAAGTTCTCACGGTACGGG
S65T pFA6a-GFP probe 10	TCCAATACATGTCCTTTCTT
S65T pFA6a-GFP probe 11	AAAAGTTTCTACTGCCCTTG
S65T pFA6a-GFP probe 12	GTTCTGTGCACGACCTCAGT
S65T PFA6A-GFP probe 13	ACTTCCACTATGGGAACAAT
S65T PFA6A-GFP probe 14	TCTTAGCTCAATTTTCCATA
S65T pFA6a-GFP probe 15	TACCTTTGTAAGAACCTGTG
S65T pFA6a-GFP probe 16	CCTTATGTTGATATTGAGTG
S65T pFA6a-GFP probe 17	TACATATGTAGTACCGTCTG
S65T pFA6a-GFP probe 18	TGTTTTCTTACCTTAGTTTC
S65T pFA6a-GFP probe 19	TCTGTGTTGTAACCTTCTACC
S65T pFA6a-GFP probe 20	CGCAAGTTGATCGTCTGGTA
S65T pFA6a-GFP probe 21	TGTTTTATGAGGTTAACCGC
S65T pFA6a-GFP probe 22	CGGGACAGGAAAATGGTCTG
S65T pFA6a-GFP probe 23	ACAGGTGTGTTAGACGGGAA
S65T pFA6a-GFP probe 24	CTTTCTAGGGTTGCTTTTCT
S65T pFA6a-GFP probe 25	CTGGTGTACCAGGAAGAACT
S65T pFA6a-GFP probe 26	AACATTGTCGACGACCCTAA
S65T pFA6a-GFP probe 27	GTACCGTACCTACTTGATAT

**Appendix Table S4. Reagents**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Pan-H3 C terminus	Millipore	Cat# 07-690 RRID:AB_417398

H3K4me2	Millipore	Cat# 07-030 RRID:AB_11213050
H3K4me3	Millipore	Cat# 05-745R RRID:AB_1587134
H3K9ac	Millipore	Cat# 07-352 RRID:AB_310544
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
SYBR green qPCR master mix	Bioline	Cat# QT605-20
CyDye™ Cy3	GE Healthcare	Cat# PA23001
Protein A Sepharose CL4B beads	VWR	Cat# 17-0780-01
<b>Critical Commercial Assays</b>		
QIAquick PCR Purification Kit	Qiagen	Cat# 28106
QIAquick Nucleotide Removal Kit	Qiagen	Cat# 28304
<b>Oligonucleotides</b>		
For Northern blotting probe primers see Table S2		
For qPCR primers see Table S1		
For RNA FISH probes see Table S3		

**Appendix Table S5. Software and Algorithms**

<b>Software and Algorithms</b>		
Fiji/ImageJ	(Schindelin et al., 2012)	<a href="https://imagej.net/Fiji">https://imagej.net/Fiji</a>
MATLAB, including Image Processing and Statistics and Machine Learning Toolboxes	The Mathworks, Inc.	
Analysis of Northern blotting and decay rates in MATLAB: display_mRNAShutdown.m	This paper	

Gillespie algorithm of RNA production and degradation written in C/CUDA: calculate_metrics.h, cuda_gillespieReduced.h, cuda_randomFuncs.h, file_funcs.h, sample_params.h, cuda_paramSearchSeparate.cu, degradation_confidenceIntervals.m	This paper	
Analysis of FISH images and quantification of RNA in Matlab: FindAndAnalyseFoci.m, DetermineCutoffs.m, DetectCellsAndQuantifyFoci.m	This paper	
MIJI		<a href="https://imagej.net/Miji">https://imagej.net/Miji</a>
CellProfiler, IdentifySecPropagateSubFunction (Matlab compiled function from Cellprofiler 1.0)	(Carpenter et al., 2006)	<a href="http://www.cellprofiler.org">http://www.cellprofiler.org</a>
Piotr's Matlab Toolbox, nonMaxSupr function		<a href="https://pdollar.github.io/toolbox/">https://pdollar.github.io/toolbox/</a>

**Appendix Table S6. Organisms/Strains**

<b>Experimental Models: Organisms/Strains</b>	
<i>S. cerevisiae</i> : Name = BY4741; Genotype = MATa; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>met15Δ0</i> ; <i>ura3Δ0</i>	Euroscarf
<i>S. cerevisiae</i> : Name = <i>GAL1:ADH1t</i> (high antisense); Genotype = <i>GAL1:ADH1t:HISMX</i>	(Murray et al., 2012)
<i>S. cerevisiae</i> : Name = TATA mutant (low antisense); Genotype = <i>GAL1:ADH1t</i> (scramble):KANMX	(Murray et al., 2015)
<i>S. cerevisiae</i> : Name = <i>GAL1:ADH1t</i> (high antisense) <i>set3Δ</i> ; Genotype = <i>GAL1:ADH1t:HISMX SET3::KANMX</i>	This study

<i>S. cerevisiae</i> : Name = TATA mutant (low antisense) <i>set3Δ</i> ; Genotype = <i>GAL1:ADH1t</i> (scramble):KANMX <i>SET3::HISMX</i>	This study
<i>S. cerevisiae</i> : Name = BY ATP4-GFP; Genotype = <i>ATP4:GFP:HISMX</i>	This study
<i>S. cerevisiae</i> : Name = BY GCV3-GFP; Genotype = <i>GCV3:GFP:HISMX</i>	This study
<i>S. cerevisiae</i> : Name = BY HMS2-GFP; Genotype = <i>HMS2:GFP:HISMX</i>	This study
<i>S. cerevisiae</i> : Name = BY TIM17-GFP; Genotype = <i>TIM17:GFP:HISMX</i>	This study
<i>S. cerevisiae</i> : Name = BY URA4-GFP; Genotype = <i>URA4:GFP:HISMX</i>	This study