Modulation of mRNA and IncRNA expression dynamics by the Set2–Rpd3S pathway

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H3K36 methylation by Set2 targets Rpd3S histone deacetylase to transcribed regions of mRNA genes, repressing internal cryptic promoters and slowing elongation. Here we explore the function of this pathway by analysing transcription in yeast undergoing a series of carbon source shifts. Approximately 80 mRNA genes show increased induction upon SET2 deletion. A majority of these promoters have overlapping IncRNA transcription that targets H3K36me3 and deacetylation by Rpd3S to the mRNA promoter. We previously reported a similar mechanism for H3K4me2-mediated repression via recruitment of the Set3C histone deacetylase. Here we show that the distance between an mRNA and overlapping IncRNA promoter determines whether Set2–Rpd3S or Set3C represses. This analysis also reveals many previously unreported cryptic ncRNAs induced by specific carbon sources, showing that cryptic promoters can be environmentally regulated. Therefore, in addition to repression of cryptic transcription and modulation of elongation, H3K36 methylation maintains optimal expression dynamics of many mRNAs and ncRNAs.
Eukaryotic transcription is regulated by post-translational modifications of histones, including acetylation, methylation, phosphorylation and ubiquitination. Histone acetylation can promote RNA polymerase II (RNAP II) transcription by disrupting the interaction between histone proteins and DNA or by recruiting factors that regulate chromatin structure and transcription. Histone acetylation is a highly dynamic modification regulated by both histone acetyltransferases and histone deacetylases (HDACs). Site-specific histone methylations also play important roles in regulating histone acetylation levels by targeting histone acetyltransferases or HDACs to specific gene locations.

The Set2 methyltransferase directly binds elongating RNAP II and co-translationally methylates H3K36 (refs 4–7). In early stages of transcription, the basal transcription factor TFIID phosphorylates serine 5 of the C-terminal domain (CTD) of Rpb1, the largest subunit of RNAP II, creating binding sites for factors involved in transcription initiation, histone modification and early termination. During transcription elongation, Cdk1 kinase phosphorylates serine 2 of CTD to create a binding site for the Set2 methyltransferase. In general, H3K36me2 and H3K36me3 are enriched in transcribed regions with increased levels towards 3′-ends, and this pattern is highly conserved in all eukaryotes. Methylated histone tails primarily function as binding sites for downstream effector proteins that have chromodomains, tudor domains or Plant Hox/PHD fingers.

Nucleosomes methylated on H3K36 are deacetylated by the Rpd3 small complex, Rpd3S (refs 15–17). The Eaf3 chromodomain and the Rec1 PHD finger mediate binding of Rpd3S to histones and this binding is stimulated by H3K36 methylation. Although Rpd3S may be in part recruited to transcribed regions via interaction with the phosphorylated CTD of RNAP II, histone deacetylation by this complex requires H3K36 methylation.

Deacetylation by the Set2–Rpd3S pathway represses transcription from cryptic internal promoters and slows transcription elongation. However, a recent study showed that the Set2–Rpd3S pathway had little effect on mRNA levels under steady-state laboratory growth conditions.

Here we describe a new function for the Set2–Rpd3S complex in modulating mRNA gene expression dynamics during induction. Microarray analysis shows that a majority of Set2-repressed genes are overlapped by long non-coding RNA (lncRNA) transcription, from either an upstream or an antisense promoter. This non-coding transcription targets H3K36me3 and the Rpd3S HDAC to the Set2-repressed promoters. In addition, new Set2–Rpd3S-repressed cryptic promoters are seen that are induced by specific carbon sources. Therefore, Set2–Rpd3S regulates both mRNA promoters and cryptic non-coding RNA (ncRNA) promoters. We previously showed that overlapping lncRNA transcription could also target H3K4me2 and the Set3C HDAC to repress mRNA induction. We find that a Set2-repressed promoter can be switched to Set3 repression by reducing the distance from the lncRNA promoter.

Results

Set2 negatively affects gene induction kinetics. To investigate how H3K36 methylation affects mRNA transcription, gene expression was analysed in set2Δ and set2Δ cells during carbon source shifts (Fig. 1a). Cells were pre-cultured in synthetic complete medium containing raffinose (0 time point) and then shifted to galactose (15, 30, 60 and 120 min), glucose (15, 30, 60 and 120 min) and then back to galactose (15 and 30 min). Under these conditions, ~800 genes were significantly induced or repressed at some point in the time course. While the HPF1 transcript levels remained steady, GAL1 and GAL7 were strongly induced in galactose media and repressed by glucose as expected (Supplementary Fig. 1). Conversely, PYK1 was downregulated in galactose media and rapidly induced during glucose incubation. In set2Δ cells, GAL1 transcript levels were slightly increased at 120 min in galactose media, while HPF1 was constitutively derepressed as previously reported. In contrast, GAL7 and PYK1 showed delayed induction in set2Δ during galactose and glucose incubation, respectively (Supplementary Fig. 1a). Therefore, Set2 plays an important role when gene expression is dynamically changing.

To further explore how Set2 affects gene expression dynamics, total RNA was analysed by high-resolution, strand-specific tiled arrays. Importantly, set2Δ did not change mRNA levels of transcriptional regulators, including GALA, GAL80, MIG1 and TUP1 (Supplementary Fig. 1b). Set2-repressed genes were identified as those showing obvious differences by visual inspection of the tiled array data with at least a twofold increase or decrease in quantitated gene expression levels at one or more time points. In total, 78 genes negatively regulated by Set2 were identified, 60 of which were induced by galactose and 18 that were constitutively expressed. For the galactose-inducible, Set2-repressed genes, the strongest differences were seen during transition periods (Fig. 1c), explaining why these 60 genes were not previously identified as Set2-regulated. Therefore, H3K36 methylation by Set2 can modulate the rate at which some genes respond to environmental changes.

Overlapping transcription targets Set2–Rpd3S to promoters. Visual inspection of the tiled array data revealed that at least 69% of Set2-repressed promoters were overlapped by RNA transcription (non-coding cryptic unstable transcripts (CUTs) or stable unannotated transcripts (SUTs), or possibly read-through transcripts from the adjacent gene) either coming from an upstream or antisense promoter. In contrast, only 12% of yeast genes overlap with lncRNA, suggesting that lncRNA transcription and Set2 might cooperate to regulate gene expression.

At AAD10 (Fig. 2a,c), YNR068C, DAL5 (Fig. 3c), SSA3 and ZRT1 (Supplementary Fig. 2a), distal upstream promoters produce a sense direction transcript that apparently continues through the open reading frame. These genes possess a second promoter, much closer to the coding region, which is derepressed by set2Δ. In contrast, the majority of Set2-repressed genes, including SUL1 (Fig. 2a), SLT1 and ARO10 (Supplementary Fig. 2a) exhibit overlapping antisense transcription. At these genes the lncRNA transcript levels were unaffected, but target mRNA transcripts were strongly increased in cells lacking SET2.

To investigate the molecular mechanism by which Set2 represses its target genes, histone methylation was analysed using data from Pokholok et al. Although H3K4me3 and H3K36me3 are typically enriched in promoters and 3′-transcribed regions, respectively, Set2-repressed genes with lncRNA transcription have non-canonical patterns (Fig. 2b). Transcription throughout the YJR154W-AAD10 locus is seen in rich media, yet Aad10 protein is not detected. An H3K4me3 peak is seen at the YJR154W promoter but not at AAD10, suggesting that the AAD10 promoter is inactive (Fig. 2b). We suspected that transcription from the YJR154W promoter produces a long RNA that overlaps the AAD10 promoter. To test this idea, we probed an RNA blot with sequences in the AAD10 gene. In wild-type cells grown in raffinose media, a single species consistent with transcription from the YJR154W promoter through the AAD10 gene was detected. In set2Δ cells, an additional short transcript initiating from the AAD10 promoter was observed (Fig. 2c). Confirming the tiled array data, loss of SET2 significantly increased AAD10 but not lncRNA transcription during galactose incubation (Fig. 2c).
Figure 1 | Set2 negatively regulates the kinetics of transcriptional induction. (a) Schematic representation of the time course experiments to determine changes in transcript levels upon carbon source shifts. (b) RNA samples from the time course experiments in (a) were analysed by high-density tiling arrays. Normalized, log₂-transformed mRNA expression levels were visualized with the Multi Experiment Viewer. + and – indicate SET2 and set2Δ, respectively. Set2-repressed genes were identified as those showing at least twofold increase in transcript levels at one or more time points. (c) Averaged profile of expression signals of 60 genes from top set in (b). (d) Averaged profile of expression signals of 18 genes from bottom set in (b). Gal, galactose; Glu, glucose; Ra, raffinose.

Prevention of H3K36 methylation by mutating H3K36 to alanine also increased AAD10 transcription (Fig. 2c). Note that the wild-type control in this experiment carries a single copy of histone H3 and H4 on a plasmid, which may account for the partially derepressed AAD10 mRNA levels.

The SUL1 gene is inactive in cells grown in rich media, but has an overlapping antisense transcript, SUT452, that is constitutively transcribed (Fig. 2a). Accordingly, H3K4me3 peaks at the 3′-end of SUL1, where the SUT452 promoter is located and H3K36me3 is enriched over the SUL1 promoter region (Fig. 2b). To test if H3K36 methylation at the SUL1 and AAD10 promoters mediate repression through the Rpd3S HDAC, chromatin immunoprecipitation (ChIP) was used to quantify histone H4 acetylation normalized to total nucleosome levels as measured by histone H3. Relative to the IncRNA promoters, the AAD10 and SUL1 promoters had reduced acetylation in wild-type cells (Fig. 2d and Supplementary Fig. 2b). Loss of SET2 increased acetylation by threefold at the mRNA promoters, but also produced a small increase at the IncRNA promoters. Eaf3 and Rco1, two components of Rpd3S, were also required for deacetylation of the AAD10 and SUL1 promoters (Fig. 2d and Supplementary Fig. 2b). Interestingly, acetylation increases were slightly higher in set2Δ than in Rpd3S mutants, suggesting additional roles for H3K36 methylation. Previous studies showed that H3K36 methylation also affects binding of a chromatin remodeler ISW1, as well as the histone chaperones, Spt6, Spt16 and Asf1, to chromatin30,31. These may also affect histone acetylation levels.

Eaf3 and Rco1 also mediated repression of the Set2-repressed genes ZRT1, STL1 and SUL1 (Supplementary Fig. 2c). Together, these results support the model that IncRNA transcription can target H3K36 methylation to mRNA promoters and thereby recruit Rpd3S to repress transcription.

Distance between promoters determines Set2 or Set3 response. We previously showed that overlapping IncRNA transcription can also target H3K4 dimethylation to mRNA promoters, recruiting the Set3C HDAC to slow the kinetics of transcription induction25. Although the Set1–H3K4me2–Set3C and the Set2–H3K36me3–Rpd3S pathways can both be targeted by IncRNA transcription, the microarray data indicates they mostly affect different genes. For example, Set3C represses DCI1 but not AAD10, while Set2–Rpd3S has the opposite pattern (Figs 2c and 3a,b). Similarly, the Set2-repressed genes YNR068C and DAL5 are not repressed by Set3 (Fig. 3c and Supplementary Fig. 3a,b). Comparing the 78 Set2-repressed genes to the 119 genes defined as Set3-repressed, there were 21 in common.

Our models predict that the distance between the IncRNA and mRNA promoters determines whether overlapping IncRNA transcription causes H3K4me2 or H3K36me2/3 to predominate at the mRNA promoter, and in turn this determines response to the two HDAC pathways. Illustrating this correlation, high
H3K36me3 is seen over the Set2-repressed AAD10, YNR068C, SUL1 and DAL5 promoters (Fig. 2b and Supplementary Fig. 3d,e), while the Set3-repressed DCI1 promoter is within a zone of H3K4me2 (Fig. 3a and Supplementary Fig. 3c). The DCI1 lncRNA and mRNA promoters are ~0.55 kb apart, while the AAD10 promoters are 1.6 kb apart.

To directly test whether altering the distance between the two promoters changes response to Set2–Rpd3S versus Set3C,
deletions of 500 or 941 bp were made between the upstream and downstream promoters at AAD10 (Fig. 4a). Northern blot analyses showed the upstream transcript with expected size change was still expressed in raffinose media, although the deletions did cause some reduction in levels or stability (Fig. 4b and Supplementary Fig. 4b). Furthermore, both the full-length and shortened upstream RNA levels decreased during galactose incubation as expected (Fig. 4b and Supplementary Fig. 4a,b).

We next examined the effects on AAD10 expression. A slight increase was seen in Δ941 cells, but AAD10 mRNA was induced by galactose similarly in wild-type and Δ500 cells (Fig. 4b). ChIP showed that shortening the distance between the two promoters alters the histone modifications. Whereas H3K36me3 was enriched at the AAD10 promoter in wild-type cells, significantly lower levels were seen in Δ941 cells (Fig. 4c). Deletion of SET2 increased histone acetylation at the AAD10 promoter in wild-type but not in Δ941 cells (Fig. 4d), as expected if reduced levels of H3K36me3 in Δ941 cells fail to recruit Rpd3S to the AAD10 promoter.

Northern blot analysis showed that deletion of SET2 slightly increased basal expression of AAD10 mRNA in raffinose media (Fig. 4e), and a similar effect was seen on deletion of the gene for the Rpd3S subunit Rco1 (Supplementary Fig. 4c). After 120 min of galactose induction, AAD10 transcript levels increased. Notably, transcription was strongly increased in either set2Δ or rco1Δ, confirming repression of the AAD10 promoter by the Set2–Rpd3S pathway. However, loss of Set2 or Rco1 had much less effect in Δ941 cells (Fig. 4e and Supplementary Fig. 4c).

To demonstrate that the effect of promoter–promoter distance on Set2-mediated AAD10 repression is not due to specific intervening...
sequences, a 941 bp of DNA fragment from a non-transcribed region of chromosome 10 (from 710,600 to 711,540) was inserted between the two promoters in Δ941 cells (designated +941, Fig. 4f and Supplementary Fig. 4d). While a DNA probe for the chromosome 10 sequence detected the lncRNA transcript only in +941 cells, a DNA probe from AAD10 sequence hybridized with lncRNA transcript in both wild-type and +941 cells (Supplementary Fig. 4d). Although deletion of SET2 had little effect on AAD10 repression in Δ941 cells (Fig. 4e), a strong increase of AAD10 transcript was seen in +941 cells lacking SET2 (Fig. 4f). This result clearly indicates that distance, and not specific DNA or RNA sequences, between the two promoters is critical for the Set2 sensitivity of AAD10.

In Δ941 cells, the distance between the upstream promoter and that of AAD10 is similar to that of the Set3-repressed DCI1 gene. ChIP experiments showed that H3K4me3 at the AAD10 promoter was low in wild-type cells, but significantly increased by Δ941 (Fig. 5a). As predicted by our models, an increase in H4 acetylation was seen upon SET3 deletion in Δ941 cells (Fig. 5b), consistent with H3K4me2 targeting Set3C to the Δ941 AAD10 promoter. Northern blots were used to determine how the H3K4me2–Set3C pathway affects AAD10 transcription. In cells grown in raffinose,
loss of Set3C (by deletion of either SET3 or the Set3C HDAC subunit HST1) showed no effect on the upstream transcript and no derepression of AAD10 (Figs 3b and 5c,e). Furthermore, wild-type cells and Set3C mutants produced similar levels of AAD10 mRNA upon galactose induction. In contrast, when the promoters are brought closer by D941, a 2.7-fold increase of AAD10 transcript was observed in the absence of Set3C (Fig. 5c), and the induction kinetics were changed (Fig. 5d). Therefore, while Set2–Rpd3S mediates AAD10 repression in its normal context, shortening the distance between the two promoters abrogates the response to Rpd3S and confers sensitivity to Set3C repression.

We sought to determine whether promoter distance correlates with which pathway mediates gene repression by overlapping transcription. Distances between promoters were plotted for the 59 Set2-repressed genes (this study) and 64 Set3-repressed genes (Kim et al., 2015), where a single ncRNA fully overlaps the mRNA promoter. Whatever average distance between the ncRNA and mRNA promoters of Set3-repressed genes was ~0.9 kb, the two promoters of Set2-response genes were on average 2.0 kb apart (Fig. 5f). These results support our proposal that distance between mRNA and ncRNA promoter determines whether Set2-Rpd3S or Set3C represses.
Inducible cryptic promoters repressed by Set2–Rpd3S. Multiple transcription elongation factors and chromatin regulators, including the Set2–Rpd3S pathway, contribute to suppression of internal cryptic promoters within mRNA genes\textsuperscript{16,21–23}. Interestingly, expression of some cryptic promoters is strongly affected by nutritional conditions\textsuperscript{22,25}. The tiling array data were analysed to determine whether any of the cryptic promoters repressed by the Set2–Rpd3S pathway respond to the carbon source shifts.

In set2\textsuperscript{Δ} cells, 118 internal cryptic promoters generating a short sense transcript and 639 cryptic promoters producing an antisense transcript were identified (Fig. 6a). There were 44 internal promoters that produce divergent cryptic transcripts, as seen for RAD28 (Fig. 6b). Remarkably, levels of at least half (416) of the Set2-repressed cryptic promoters were regulated by specific carbon sources (Fig. 6a). Representative genes SLD3, TMA108 and RAD28 are shown in Fig. 6b, none of which are derepressed by deletion of SET3 (ref. 25; Supplementary Fig. 5). The cryptic transcripts within SLD3, TMA108 and RAD28 increased in galactose media, indicating that their internal promoters require some additional activation signal beyond the absence of Set2.

Microarray and northern blots showed at least three cryptic promoters within the PCA1 gene that are repressed by the Set2–Rpd3S pathway (Fig. 6c). Interestingly, these cryptic promoters differentially respond to galactose. The 5'–cryptic promoter (No. 1) is constitutively active, while the middle cryptic promoter (No. 2) is downregulated and the 3'–promoter (No. 3) is induced during galactose incubation. Furthermore, the 3'–cryptic promoter appears to be bidirectional as antisense transcripts are also increased. These results show that cryptic promoters repressed by the Set2–Rpd3S pathway differentially respond to environmental signals, and it will be interesting to decipher how this regulation is carried out.

**Discussion**

The Set2–Rpd3S pathway has been shown to suppress cryptic internal transcription initiation sites, but a role in regulating

![Figure 6](https://example.com/figure6.png)

**Figure 6 | Set2 represses inducible cryptic promoters.** (a) Tiling array data identified 757 cryptic transcripts increased in set2\textsuperscript{Δ}. Venn diagram shows the number of genes with internal cryptic promoters repressed by Set2: 639 antisense and 118 sense direction. Among these, 55% (416 promoters) are more active during carbon source shifts in the absence of SET2, indicating that they are inducible cryptic promoters responding to environmental changes. In all, 44 cryptic promoters repressed by Set2 produce both sense and antisense divergent transcripts. (b) Examples of distinct cryptic promoter types. Red arrows and blue arrows show the core promoters and cryptic promoters, respectively. Light blue boxes show the open reading frame position. SLD3 or TMA108 has an internal cryptic promoter or an antisense promoter that is activated during galactose incubation. RAD28 gene has internal cryptic promoters that produce short sense and antisense transcripts. (c) Northern blot analysis of PCA1 cryptic transcripts. SET2 or set2\textsuperscript{Δ} cells were grown in synthetic complete (SC) medium containing raffinose (Ra) and shifted to SC-galactose media for 120 min (Gal120). WT, wild type.
mRNA expression has been elusive. Mutants in this pathway have only small effects on steady-state transcript levels\(^{24}\). Here we show that Set2–Rpd3S affects the dynamics of gene expression at a subset of promoters, delaying or repressing transcriptional activation upon carbon source shifts (Fig. 1b,c). Although deletion of Set2 increased basal transcript levels at some genes, differences were generally greatest during carbon source transition periods. Importantly, most Set2-repressed promoters have overlapping lncRNA transcription from either an upstream promoter or downstream antisense promoter. This transcription, independently of the ncRNA itself, deposits H3K36 methylation over the Set2-repressed promoter, leading to histone deacetylation by Rpd3S (Fig. 7a). This targeted deacetylation slows gene induction, presumably creating a requirement for a stronger or longer duration inducing signal. This type of kinetic regulation is likely to be critically important for cells growing in the wild, where they must continuously alter gene expression patterns to adapt to changing environmental conditions.

Several previously described examples of repressive ncRNA expression fit this model. IME1, encoding a master regulator of meiosis, is repressed by expression of the lncRNA IR71 in cis. IR71 transcription brings Set2 and Set3C to the IME1 promoter and blocking IR71 transcription alleviates IME1 repression\(^{32}\). IME4, another key regulator of meiosis, is also repressed by antisense transcription\(^{33}\). In the absence of Set2, this gene was slightly derepressed in galactose-grown cells. Recently, Nguyen et al.\(^{34}\) studied genes differentially expressed in glucose and galactose, and showed that many of these have promoters with overlapping transcription. For example, HMS2 transcription negatively impacts the overlapping antisense RNA SUT650 and the downstream gene BAT2 (ref. 34). Interestingly, we see that BAT2 and SUT650 are Set2-repressed, suggesting that read-through transcription from HMS2 targets Set2–Rpd3S to the BAT2 promoter. Set2 effects are also seen at several other loci studied by Nguyen et al., including BAG7 and HIS1/GIP2. The ZRT1 gene, with a distal upstream promoter and proximal promoter closer to the open reading frame, is also derepressed in set2A and mutants for Rpd3S (Supplementary Fig. 2a,c). A previous study showed that insertion of transcription terminators between the distal and proximal promoters derepressed ZRT1 transcription\(^{35}\). The behaviour of all these genes can be explained by overlapping lncRNA transcription targeting H3K36 methylation and Rpd3S deacetylation to the core promoters (Fig. 7a).

Interestingly, we discovered many inducible cryptic promoters repressed by the Set2–Rpd3S pathway that were not identified in earlier studies done under steady-state conditions (Fig. 6a). One previous study found that 1,685 genes had increased acetylation within coding regions in set2A, but only 56% of these produced detectable internal cryptic transcripts\(^{33}\). The remaining genes may lack appropriate basal promoter elements for cryptic transcription. Alternatively, any cryptic transcripts produced at these genes in Set2–Rpd3S mutants may be rapidly degraded by nuclear exosome or other nucleases. For example, antisense transcripts are seen at STE11 or CTT1 in a roa1Δrpp6Δ strain, but not in roa1Δ (ref. 36). Our results suggest one additional possibility: these genes may contain cryptic promoters that are only activated transiently or

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**Figure 7 | Models for regulation of gene expression by Set2–Rpd3S pathway and overlapping lncRNA transcription.** (a) At Set2-repressed promoters, transcription from a distal or antisense promoter targets H3K36me3 to the core promoter of mRNA target genes. Rpd3S deacetylates histones in the mRNA promoter region, resulting in delayed or reduced induction. For suppression of internal cryptic promoters, transcription from the mRNA promoter of mRNA genes targets H3K36me3 and Rpd3S deacetylation in 3′-transcribed regions. These cryptic promoters may also respond to environmental changes. (b) Position of the lncRNA promoter specifies the repressive effects of two distinct HDACs, Set3C and Rpd3S. H3K4me2 and H3K36me3 are targeted to 5′- and 3′-regions of lncRNA expression, respectively. In 5′-regions, Set3C binds to H3K4me2 to deacetylate histones and repress the mRNA promoter. In contrast, in 3′-regions of lncRNA transcription, Rpd3S binds to H3K36me3 to deacetylate histones and repress mRNA promoters. These mechanisms are also applicable to genes with antisense transcription.
under certain conditions. In our study, ~55% of Set2-repressed 
cryptic promoters were most active during carbon source shifts 
(Fig. 6a), indicating these cryptic promoters are responding to 
environmental signals, as well as to loss of the Set2–Rpd3S 
pathway. As these responses are often different from the coupled 
DNA promoter, it will be interesting to figure out where the 
relevant regulatory elements reside.

It should be noted that increased internal cryptic initiation may 
have affected earlier genome-wide microarray analyses that used a 
limited number of DNA probes, as 3′-probes can hybridize with both 
full-length mRNA and short cryptic transcripts. For example, 
PCA1 and STE11 are annotated as Set2-repressed genes 
based on microarrays16,21,24. However, their full-length mRNAs 
are unaffected in Set2–Rpd3S mutants (Fig. 6c). The increase in 
signal is due to sense-strand internal cryptic transcripts that are 
derepressed by a SET2 deletion.

Another important finding of this study is that Set2-mediated 
effects seen in the first galactose incubation are generally not 
observed during the second galactose pulse (Fig. 1c). This pattern 
was also observed for Set3C-mediated delays of gene induction25. 
These results suggest a model in which chromatin regulation by 
nRNA transcription could contribute to ‘memory’ of recent gene 
expression. Set2-repressed promoters that have not been 
expressed recently tend to have high levels of H3K36me3 and 
low acetylation due to the overlapping nRNA transcription. 
During the first galactose induction the mRNA promoter marks 
will be replaced with H3K4me3 and high acetylation. Even if 
mRNA transcription levels have returned to repressed levels after 
two additional hours in glucose media, H3K36 methylation may 
not yet have been re-established over the mRNA promoter 
(perhaps because the relevant nRNA promoter has now itself 
repressed by the overlapping coding RNA transcription). 
Therefore, the second galactose induction would not need to 
overcome Set2–Rpd3S repression and the response will be more 
rapid. In cells lacking Set2 or Rpd3S, the kinetics of the first 
induction are more similar to the second.

There are obvious parallels between Set2–Rpd3S and Set3C in 
mediating repression by overlapping nRNA transcription. It is 
therefore pertinent to ask whether the two pathways operate 
independently. Our data indicate that the majority of genes and 
cryptic transcripts repressed by Set2 are not affected by deletion of 
SET3, and vice versa. Response appears to correlate with the 
relative levels of H3K4me2 or H3K36me3, the respective localizing 
marks for Rpd3S and Set3C HDACs (Fig. 7b). This is most clearly 
demonstrated by experiments reducing the distance between 
AAD10 and a repressive upstream promoter. The Δ941 deletion 
changes the methylation pattern at AAD10, abrogating Set2–Rpd3S 
repression while making the promoter sensitive to Set3C (Figs 4 
and 5). There is a subset of promoters responsive to both pathways, 
as previously reported for IME1, presumably because of the 
overlap between H3K4me2 and H3K36me3 at these loci32.

The roles of cryptic transcription and its nRNA products are 
not yet fully understood. Previous studies show that some cryptic 
transcripts can be translated into short proteins12,37. There are 
also cases where the nRNA can serve as a scaffold or for 
targeting bound protein complexes38. We have shown in this 
report and in Kim et al.25 that overlapping nRNA transcription, 
independent of the nRNA itself, can also target histone 
modifications to mRNA core promoters to modify transcription 
responses. Given the plethora of ncRNAs with no clear function, 
these types of cis-mediated chromatin effects may be more 
common than previously appreciated.

Methods

Yeast strains and plasmids. Yeast strains and plasmids used in this study are 
listed in Supplementary Table 1. The time course experiments were done with 
matched SET2 and set2A strains as described in the text and as previously 
published for Set3 analysis25. To generate Δ580, Δ941 and + 941 strains in Fig. 4, 
the delitto perfetto strategy was used25. Briefly, for the Δ580 and Δ941 strains 
the CORE cassette containing KanMX4 and KIURA3 was inserted between the two 
promoters of AAD10. The resulting strain was transformed with oligonucleotides that 
delete the upstream regions of AAD10 while also removing the CORE markers. 
To generate + 941 strains, the CORE cassette was inserted between the two 
promoters in Δ941 cells and replaced with a 941 bp of DNA fragment from a non-
transcribed region of chromosome 10.

Reverse transcription–PCR. RNA was extracted from cells with hot phenol. 
First-strand cDNA was prepared with 1 μg total RNA. Superscript II reverse 
transcriptase (Invitrogen) and gene-specific primers. For Supplementary Fig. 1a, one-fifteenth of the cDNA and 0.6 μCi [α-32P]dATP were used for PCR amplification, 
which consisted of 60 s at 94°C, followed by 23–26 cycles (determined to be 
in the linear range of amplification for each primer pair) of 30 s at 94°C, 30 s at 
55°C and 45 s at 72°C, followed by 2 min at 72°C. PCR signals were quantitated 
by Fuji PhosphoImager. For Supplementary Fig. 2d, cDNA was analysed by real-
time quantitative PCR using SYBR Green Supermix and CFX96 cycler (Bio-Rad). 
The sequences of oligonucleotides used in this study are listed in Supplementary 
Table 2.

Northern blot analysis. RNA was isolated from cells with hot phenol and 10 μg 
total RNA from each sample was separated on an agarose gel, blotted to 
membranes and hybridized to radioactive probes using standard techniques40. 
The sequences of oligonucleotides used for northern blot analysis are listed in 
Supplementary Table 2. Strand-specific probes were generated by unidirectional 
PCR in the presence of [α-32P]dATP with only one primer. Hybridization was 
done in a buffer containing 1% BSA, 7% SDS, 1 mM EDTA 
(pH 8.0) and 300 mM sodium phosphate buffer (pH 7.2).

Chromatin immunoprecipitations. ChIPs were done as previously described with 
the modifications described below41. The following histone antibodies were used: 
1 μl of anti-H3K4me2 (Upstate 07-030); 0.5 μl of anti-H3K36me3 (Abcam 90503); 
0.5 μl of anti-acetyl H4 (Upstate 06-598); and 2 μl of anti-H3 (Abcam 1791). 
All antibodies were bound to Protein A-agarose beads and used for precipitation of 
formaldehyde-crosslinked chromatin. For anti-H3 antibody, binding was done in 
FA lysis buffer containing 275 mM NaCl. For other antibodies, binding was done in 
FA lysis buffer containing 1 M NaCl. Precipitated DNAs were analysed in real-time 
using SYBR Green Supermix and CFX96 cycler (Bio-Rad). Oligonucleotides for PCR 
analysis are listed in Supplementary Table 2.

High-resolution tiling arrays. High-resolution tiling arrays were done as pre-
viously described25. Briefly, RNA isolated from cells was converted to cDNA with 
a combination of random and oligo dT priming, labelled and hybridized to tiled 
Affymetrix arrays carrying the yeast genome. Data are plotted in in42 and 
can be visualized with a browser at http://stemmetlab.embl.de/buratowski Labs/index.html. 
The raw microarray data can be downloaded from ArrayExpress with the accession code E-MTAB-4268.

Data availability. Microarray data sets that support the findings of this study have 
been deposited in the ArrayExpress repository (https://www.ebi.ac.uk/arrayexpress/) 
with the accession code E-MTAB-4268.

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Erratum: Modulation of mRNA and IncRNA expression dynamics by the Set2–Rpd3S pathway

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The ArrayExpress accession code is incorrect in this Article. The correct accession code is E-MTAB-4268.