# Making a Noisy Gene: HDACs Turn Up the Static

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**In this issue of *Molecular Cell*, Weinberger et al. (2012) find that particular histone deacetylases (HDACs) regulate distinct stages of transcription, implicating chromatin dynamics in the generation of gene-specific noise within populations of genetically identical cells.**

Gene expression in genetically identical cells growing in common environments can show stochastic variations over time, a phenomenon known as gene expression noise. While early investigations in this area focused on identifying and distinguishing between different sources of noise within a cell (Blake et al., 2003, 2006; Elowitz et al., 2002; Ozbudak et al., 2002), more recent work has made clear that noise levels can vary widely between individual genes (Suter et al., 2011). Promoter sequence, nucleosome occupancy, and the presence of chromatin regulators have all been identified as factors contributing to or buffering gene expression fluctuations and phenotypic variability (Blake et al., 2006; Suter et al., 2011; Whitelaw et al., 2010). In this issue of *Molecular Cell*, Weinberger et al. (2012) build on these findings and show that certain chromatin regulators can act to increase noise levels at their target genes.

Eukaryotic genes are exquisitely compacted into chromatin by interactions with histone proteins, which condenses them by many orders of magnitude through organization into nucleosomes and higher-order structures. Transcription of these genes occurs in staccato bursts, in which multiple messenger RNA molecules are synthesized from a template DNA strand, and comprises multiple steps including recruitment of the preinitiation complex, initiation of transcription, and elongation by RNA polymerase. All of these steps represent potential points of transcriptional regulation, and chromatin modifiers that control the packaging and accessibility of DNA play a key role in regulating these steps. By screening for the effect of deleting particular chromatin regulators in the yeast *Saccharomyces cerevisiae* and measuring the effect on gene expression noise and levels of reporter constructs driven by a library of promoters, Weinberger et al. (2012) identified chromatin regulators that can modulate expression levels and noise by controlling transcription at particular steps.

The authors screened individual deletions of 137 chromatin factors using flow cytometry of a library of promoter constructs spanning a range of expression levels and used a theoretical model to infer regulatory mechanism from the relationship between noise and mean expression. By obtaining single-cell measurements of reporter gene expression in the individual deletion constructs, they were able to deconvolute the effect of loss of a given gene on transcriptional bursting of the reporter and identify independent modulators of burst size and burst frequency. Deletions that affected transcriptional burst size changed mean expression levels without altering noise levels. By contrast, deletions that altered the frequency of bursting resulted in changes to both mean expression and noise levels (Figure 1).

Perturbations to both histone H2B monoubiquitination (ubH2B) and the histone deacetylase (HDAC) complex Set3C resulted in increases in transcriptional burst size, implicating these pathways as repressors of burst size (Weinberger et al., 2012). Deletions of histone acetyltransferases (HATs) decreased the frequency of transcriptional bursts while removing other HDACs, including members of the Rpd3(L) complex, increased burst frequency (Figure 1). While both Set3C and Rpd3(L) seemed to act as transcriptional repressors, the distinct noise phenotypes of knocking out particular HDAC complexes implied that these factors are specialized to regulate distinct steps of transcription, which the authors went on to investigate further. By examining published genome-wide occupancy profiles of RNA polymerase II (Pol II) subunits and elongation factors, they found that ubH2B and Set3C targets were associated with low levels of Pol II processivity, suggesting that these factors act during elongation to increase the chances of abortive transcription. Correlation of ubH2B occupancy with low levels of histone acetylation, along with an increase in acetylation at Set3C targets in strains deleted for H2B ubiquitinating enzymes, supports a model in which ubH2B acts to recruit Set3C to target genes. By mapping levels of the H3K9 acetylation (H3K9ac) mark, which tends to be positively associated with gene expression, in strains knocked out for Set3C or Rpd3(L) components, the authors defined a role for Rpd3(L) in deacetylating the +2 nucleosome downstream of the transcriptional start site. Earlier work had found an association between nucleosome occupancy at genes and high noise levels (Cairns, 2009). Deacetylation of the +2 nucleosome by Rpd3(L) presumably decreases the probability of transcriptional initiation, following recruitment of the transcription apparatus to the promoter.

Functional specialization of HDACs provides an opportunity for evolution to fine-tune and sculpt distinct parameters of gene expression programs. Modulating Set3C or ubH2B activity at a given gene could alter the probability of full-length transcription and change mean

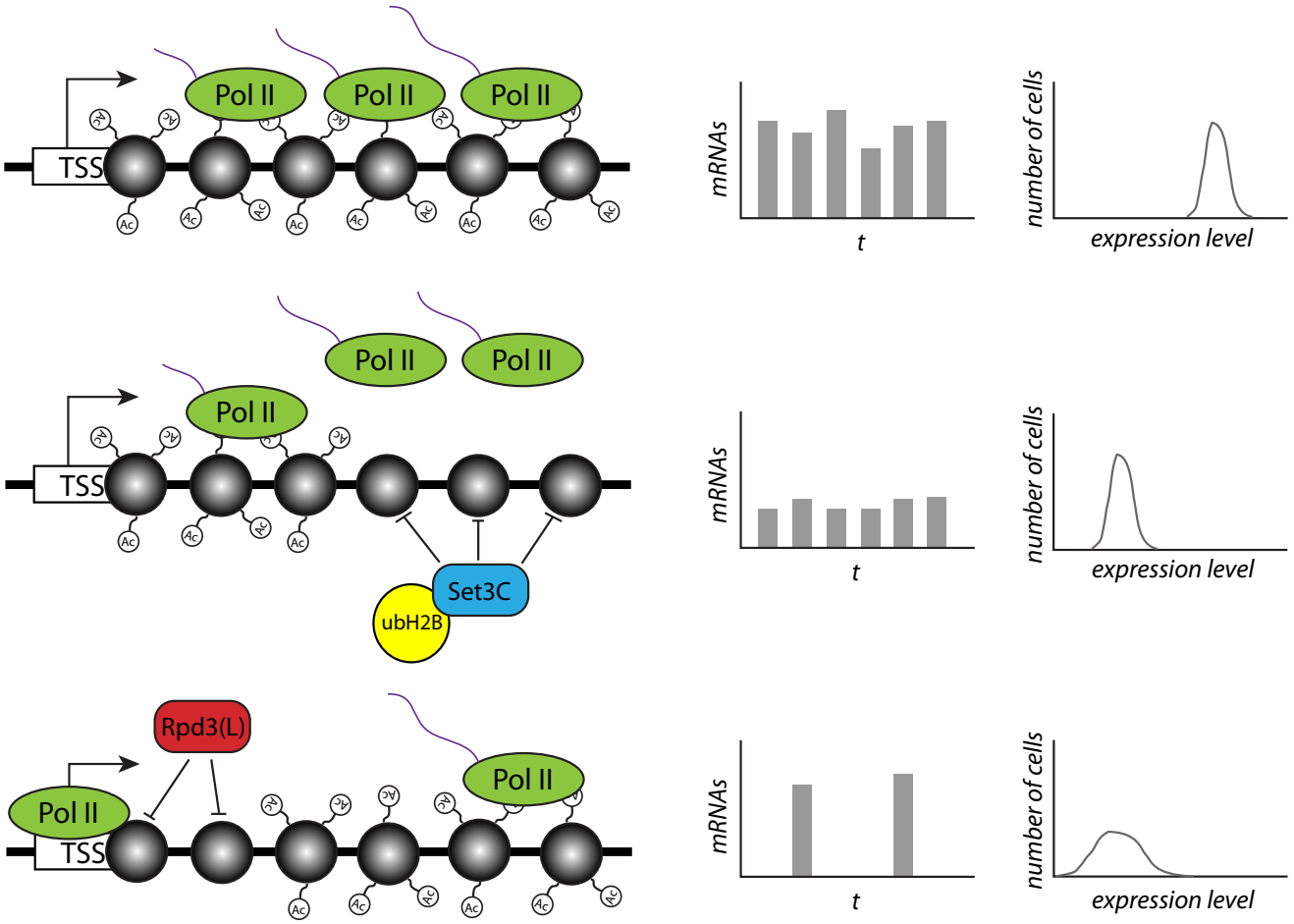


**Figure 1. The HDACs Set3C and Rpd3(L) Act at Distinct Steps of Transcription to Modulate Either Transcriptional Burst Size or Burst Frequency**

The figure illustrates the effect of histone deacetylation by either Set3C or Rpd3(L) on the expression and noise levels of a target gene. In the top row, RNA polymerase II (Pol II) transcribes RNA (purple strands) from a gene with heavily acetylated nucleosomes (gray circles), leading to high-frequency transcriptional bursts of relatively large size. When Set3C and ubH2B act to deacetylate nucleosomes in the body of the gene (middle row), it causes a decrease in Pol II processivity and an increased likelihood of abortive transcription. This affects the average size of each transcriptional burst, but not the frequency, resulting in a decrease in mean expression levels but the same level of noise. Rpd3(L), by contrast, acts to deacetylate nucleosomes just downstream of the transcription start site (TSS), leading to a reduced frequency of transcriptional initiation and bursts (bottom row). This lower burst frequency results in both lower mean expression levels and increased noise levels, as seen by the increased width of the expression distribution.

expression levels, while changing Rpd3(L) activity could instead adjust the frequency of transcriptional bursting and increase or decrease noise levels. Frequency-modulated signal encoding has been recognized as a regulatory strategy used by biological systems (Locke et al., 2011), and it is conceivable that transcriptional bursting frequency could play a broader role in controlling other biological processes. Regulation of transcriptional elongation is an emerging theme in many areas of biology, from embryonic fly development to mammalian stem cell fate choice (Core and Lis, 2008). Control at this postinitiation step of transcription may be advantageous to biological systems as it offers the potential for faster and more synchronous regulation in response to signaling factors through control of pause release. Surprisingly, the principal modulators of burst size identified by Weinberger et al. (2012) act at the level of elongation rather than at earlier steps of transcription, indicating that Pol II processivity may be an actively regulated process. Importantly, the approach taken here allows for dissection of regulation at distinct steps of transcription.

While it is clear that different genes can exhibit unique noise profiles and transcriptional bursting kinetics, the functional consequences of gene-specific noise remain largely unknown. Might there be particular genes, environmental conditions, or life cycle stages at which regulation of noise levels is particularly advantageous? Just as it is widely recognized that mean gene expression levels respond to environmental stimuli and developmental cues, noise levels, burst size, and burst frequency also represent distinct parameters that could potentially be manipulated to govern cell behavior (Blake et al., 2006; Locke et al., 2011; Weinberger et al., 2005). In the postgenome age, a great deal of effort has gone into understanding the changes in

mean gene expression levels that occur as cells change state. Deciphering the structure of gene expression noise and dynamics in the context of biological programs represents an emerging frontier in understanding how evolution shapes transcriptional programs, and it will be interesting to see how these concepts extend to metazoan cells.

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# Should I Stay or Should I Go? Chromodomain Proteins Seal the Fate of Heterochromatic Transcripts in Fission Yeast

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**In this issue of *Molecular Cell*, Ishida et al. (2012) and Keller et al. (2012) show distinct outcomes for heterochromatic RNAs that bind different chromodomain proteins; Chp1 tethers transcripts to centromeres, whereas Swi6$^{HP1}$-bound transcripts are evicted from chromatin and destroyed.**

Posttranslational modification of histones and the proteins that recognize these changes coordinate the arrangement and utilization of chromatin. Heterochromatin is characterized by transcriptional silencing, histone hypoacetylation, and enrichment for methylation on K9 and K27 of histone H3. Silencing can occur via reduction in RNA polymerase access (transcriptional suppression) and through posttranscriptional destruction of RNA. Chromodomain proteins bind to H3K9me2/3- or K27me3-marked chromatin and recruit additional chromatin regulatory complexes that can reinforce and spread the heterochromatic signals. Despite being among the earliest-identified and best-known nonhistone proteins associated with heterochromatin, the mechanistic details of how chromodomain proteins such as HP1 (heterochromatin protein 1) act to silence transcription are poorly understood. Keller et al. (2012) provide evidence that the fission yeast HP1 homolog Swi6 directly captures heterochromatin-associated transcripts and targets them for degradation.

The fission yeast *S. pombe* harbors four chromodomain proteins that are known to recognize H3K9 methylation, including two HP1-like proteins, Swi6$^{HP1}$ and Chp2$^{HP1}$, which have largely nonoverlapping activities that contribute to heterochromatic silencing. Chp2$^{HP1}$ acts to silence heterochromatin at the transcriptional level. In contrast, Swi6$^{HP1}$ has been implicated as associating with a variety of chromatin-modifying factors and, unlike Chp2$^{HP1}$, associates with RNA in vivo. Swi6$^{HP1}$ is presumed to be largely involved in the co- or posttranscriptional processing of heterochromatic transcripts rather than acting as a barrier to prevent transcription itself (Motamedi et al., 2008).

Until recently, it was not known whether Swi6$^{HP1}$ associates with RNA directly or through an intermediary protein. Keller et al. (2012) demonstrated that recombinant Swi6$^{HP1}$, like HP1 isoforms in other organisms (Muchardt et al., 2002), binds RNA directly in vitro primarily through the hinge domain, with contributions from other regions including the chromodomain. Mutation of positively charged residues within the hinge (Swi6-KR25A) disrupted the association of RNA with Swi6$^{HP1}$ in vivo. The mutant protein was still capable of being recruited to heterochromatin by its affinity for H3K9