association of RBPs with GREs ultimately determines the half-life of the mRNA and/or its translation. Within the framework of the study, it will also be interesting to investigate whether T cell activation affects the interaction of CUGBP1 and GRE-containing mRNAs: do RNP complexes increase, decrease, or change subcellular location? Broader questions center on the elucidation of the degradation machineries responsible for the breakdown of GRE-containing mRNAs: Are the exosome, the proteasome, or processing bodies implicated or is there a specialized GRE-mRNA-degrading apparatus? Is CUGBP1-triggered deadenylation required for the decay of GRE-containing mRNAs?

As examples accumulate of both ARE-bearing stable mRNAs and labile mRNAs lacking AREs, the ARE dogma has incrementally given way to alternative bona fide instability sequences. In this context, the novel GRE degradation motif identified by Vlasova et al. (2008) provides the fast-advancing field of accelerated mRNA decay with broader insight into the nature of turnover determinants. With an increasing understanding of mRNA regulatory elements and the mRNA-binding factors (RBPs, microRNAs) that interact with them, the 3′UTR emerges as an ever richer platform from which to govern gene expression.

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Dubbing SAGA Unveils New Epigenetic Crosstalk

W.W.M. Pim Pijnappel[1] and H.Th. Marc Timmers[1,*]
[1]Department of Physiological Chemistry, STR3.201, University Medical Centre Utrecht, Universiteitsweg 100, 3508 AB UTRECHT, The Netherlands
*Correspondence: h.t.m.timmers@umcutrecht.nl
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In a recent issue of *Molecular Cell*, two independent studies (Zhang et al., 2008; Zhao et al., 2008) provide compelling evidence that targeted deubiquitylation of histones is intimately linked to transcription activation, epigenetic regulation, and cancer progression.

The yeast SAGA coactivator complex has served as a paradigm for the interconnection between chromatin modification and the transcriptional status of genes (Lee and Workman, 2007). A plethora of genetic and biochemical studies showed that SAGA connects gene-specific activator proteins, the basal RNA polymerase II-transcription machinery, and histone acetylation, which represents an activating chromatin mark (Berger, 2007). More recently, yeast SAGA was shown to harbor the Ubp8p deubiquitylase (DUB) enzyme (Berger, 2007). Two recent studies on the metazoan orthologs provide new insight into histone crosstalk.

The multigroup effort headed by Didier Devys identifies three new subunits of the TFTC/STAGA complexes in *Drosophila melanogaster* and in human cells (Zhao et al., 2008) and an overall composition that is very similar to yeast SAGA. Therefore, we believe that it is timely to abandon the "old" TFTC/STAGA/PCAF names now and to embrace SAGA as the descriptor for orthologous complexes in other organisms. The three new SAGA subunits are human USP22 (Ubp8p in yeast or Nonstop in flies), ATXN7L3 (ySfg11p or dSgf11), and ENY2 (ySus1p or dE[y]2). Zhao et al. (2008) show that the new members form a SAGA submodule with TAF5L and ATXN7. Purified human SAGA, but not the recombinant submodule, can remove ubiquitin efficiently both from histone H2A and H2B in vitro. Whereas monoubiquitylated H2B (H2Bub1) is linked to transcription elongation (Wyce et al., 2007) and is required for methylation of K4 and K79 of histone H3 in yeast, H2Aub1 is metazoan specific and formed by the action of Polycomb repressive complex 1 (PRC1), a regulator of facultative heterochromatin (Berger, 2007). Using the position effect variegation (PEV) assay for facultative heterochromatin in fruit flies, they find that dNonstop and dSgf11 mutations enhance the variegated

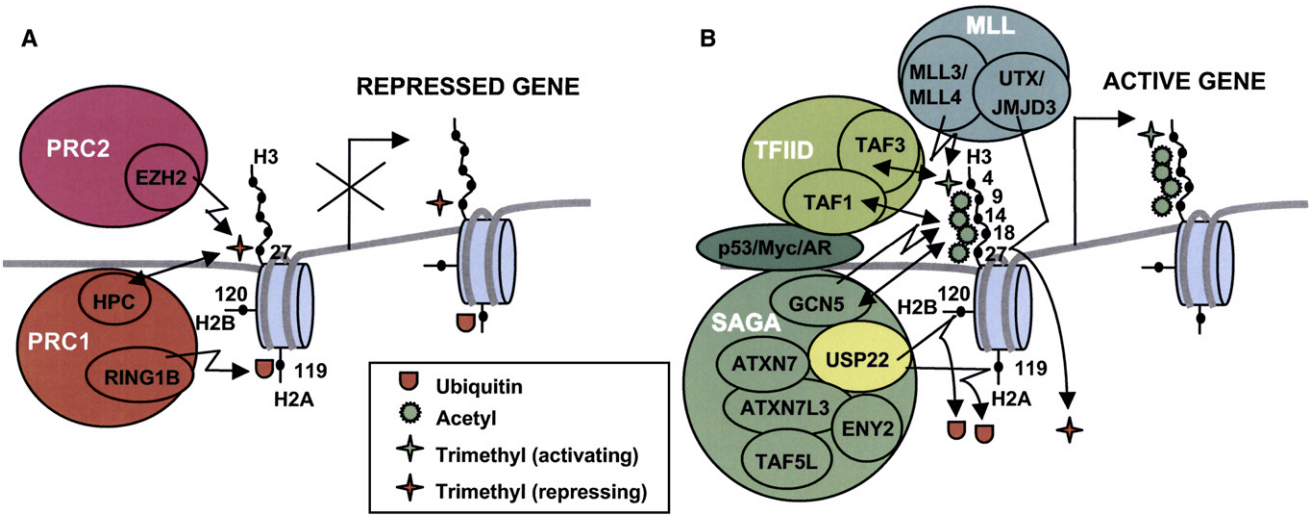

Figure 1. Model for DUB Action in Gene Activation

Model describing the possible interplay of histone modifications around USP22 and H2A monoubiquitylation relevant for transcriptional repression (A) or activation (B). Angled arrows indicate enzymatic activity. Double-headed arrows depict binding activity.

phenotype indicative of *white* gene repression, whereas dNonstop overexpression activates transcription. Interestingly, the PEV function does not require the dNonstop ZnF domain, which is essential for USP22 integration into SAGA. Additional experiments show that both hSAGA and dSAGA can be recruited to promoters by the androgen receptor (AR) and that full activation of AR target genes is regulated by hUSP22 and dNonstop expression.

The starting point for the other study (Zhang et al., 2008) was the identification of the USP22 ubiquitin hydrolase as a part of a cancer stem cell signature, which also includes two Polycomb group members, BMI-1 and RNF2/Ring1b. Interestingly, BMI-1 and RNF2/Ring1b form the E3 ligase that directs H2A ubiquitylation. Using conditional expression of the c-*myc* and p53 transcriptional activators, Zhang et al. (2008) find that USP22 knockdown reduces transcriptional activation of all c-*myc* and of most p53 target genes tested. As expected for a "true" coactivator, USP22 recruitment to target promoters depends on c-*myc*, but c-*myc* recruitment is not dependent on USP22. Knockdown of USP22 compromises cell-cycle progression and anchorage-independent growth, which strengthens the link with cancerous growth. By epitope tagging, Zhang et al. (2008) also find that USP22 is stably associated with multiple subunits of SAGA and that USP22-purified SAGA can both acetylate histones and deubiquitylate H2Bub1 in vitro. Although they did not test H2Aub1 as a substrate, this was clearly demonstrated by Zhao et al. (2008). Unfortunately, neither study tested ubiquitylated nucleosomes as DUB substrates. USP22 might have a broad substrate specificity, as its initial characterization (Lee et al., 2006) showed that it can also utilize an artificial substrate (ubiquitin/β-galactosidase fusion).

These two papers put histone (de)-ubiquitylation in the spotlight of epigenetics and cancer biology and have important ramifications for follow-up studies. Important questions for USP22 and other histone DUBs (Nakagawa et al., 2008; van der Knaap et al., 2005) include the following:

- How can histone substrate specificity of DUBs be validated in vivo?
- Do histone DUBs have additional substrates relevant for chromatin and transcription?
- How are the DUBs targeted to chromatin loci?
- How are the H2Aub1 and H2Bub1 marks "read"?

For USP22, several observations indicate that targeting occurs via SAGA recruitment. However, the PEV function of USP22 does not seem to require SAGA incorporation (Zhao et al., 2008). Possibly, this DUB can also act outside of its SAGA context. The fact that USP22 is the only SAGA subunit in the cancer stem cell signature hints at this possibility. Related to the DUB specificity question is identification of protein (complexes) "reading" the ubiquitin-histone code. Although no direct binders of H2Aub1 or H2Bub1 have been identified yet, recent data show that H2Aub1 reconstituted chromatin is a poor template for in vitro transcription (Nakagawa et al., 2008), which might relate to the inability of the histone H3K4 methyltransferase MLL3 (also called KMT2C) to use H2Aub1 nucleosomes.

Together with this finding, the two new studies (Zhang et al., 2008; Zhao et al., 2008) suggest that crosstalk between histone modifications like H3 methylation/acetylation and H2Aub1 could depend on the interplay between the Polycomb group, SAGA, and MLL3(KMT2C)/MLL4(KMT2D) histone-modifying complexes (Figure 1). To maintain the transcriptionally repressed state, the PRC1 and PRC2 complexes ubiquitylate H2A at K119 and trimethylate H3 at K27, respectively. These modifications prevent H3K4 methylation (Nakagawa et al., 2008) and acetylation at H3K27. Signal-dependent activation by sequence-specific transcription activators would recruit SAGA to remove ubiquitin from histones via USP22. This action would allow the MLL complexes to trimethylate H3K4, which stabilizes TFIID binding via the TAF3-PHD (Vermeulen et al., 2007). The MLL3/MLL4 complexes also harbor

H3K27 demethylases (Rivenbark and Strahl, 2007), and would liberate H3K27 for subsequent SAGA-dependent acetylation. In this pathway SAGA not only plays a pivotal role in gene induction but also collaborates with MLL complexes to counter Polycomb-mediated repression and to maintain gene expression programs relevant for cancer via epigenetic mechanisms.

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Linking Differential Chromatin Loops to Transcriptional Decisions

Effie Apostolou[1] and Dimitris Thanos[1,*]
[1]Institute of Molecular Biology, Genetics and Biotechnology, Biomedical Research Foundation, Academy of Athens, 4 Soranou Efesiou Street, Athens 11527, Greece
*Correspondence: thanos@bioacademy.gr
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GATA-1 and GATA-2 control proliferation and differentiation of hematopoietic progenitor cells via transcriptional regulation. In this issue of *Molecular Cell*, Jing et al. (2008) demonstrate that GATA factor exchange on the *Kit* locus directs a transcriptional switch by reconfiguring chromatin loops.

Hematopoiesis is a classical model system to study the regulation of gene expression during development and differentiation. During differentiation, hematopoietic stem cells progressively lose their self-renewal capacity and mature to specialized blood cells that produce specific proteins, including the hemoglobins. The GATA family of transcription factors is made of key regulators of hematopoiesis; they regulate the expression of genes involved both in the proliferation of progenitor cells and their differentiation to mature blood cells (Cantor and Orkin, 2005; Kim and Bresnick, 2007). GATA-1 is critical for terminal differentiation and maturation of progenitor cells, whereas GATA-2 is required for the maintenance and proliferation of the hematopoietic progenitor cells. Furthermore, both factors are positively autoregulated, GATA-1 represses the expression of GATA-2, whereas GATA-2 activates GATA-1 expression, thus providing evidence for a regulatory network of GATA factors controlling hematopoiesis (Ferreira et al., 2005).

GATA-1 and GATA-2 possess similar intrinsic abilities to bind GATA sites and interact with other transcriptional regulatory proteins, (e.g., nucleosome modifiers, cofactors, etc.), including the specialized GATA cofactor FOG-1 (Rodriguez et al., 2005). GATA-FOG-1 interactions are critical for GATA function in vivo, but their mechanism of action remains unknown. In addition, FOG-1 bound to GATA factors can have opposite effects in regulation of transcription (i.e., activation versus repression) in different promoter and enhancer contexts (Cantor and Orkin, 2005). Most likely, the geometry of the GATA-FOG-1 complex, when bound to different sites, is influenced by the precise DNA sequence and/or by nearby factors. Therefore, the complex might undergo allosteric changes, leading to the recruitment of coactivator or corepressor complexes. The hallmark of GATA-1 and other erythroid-specific transcription factor (e.g., EKLF) function during erythropoiesis is their involvement in the formation of chromatin loops between the remote globin enhancer (locus control region [LCR]) and individual globin gene promoters in a competitive manner (Vakoc et al., 2005). These chromatin loops are formed between the LCR and the relevant globin gene in a developmentally regulated manner and have been correlated with gene activation. However, it remains unclear whether chromatin loops supporting the expression of a gene can be reconfigured when this gene is turned off and what the role, if any, is of transcription factors in specialized chromatin loop configurations.

In this issue of *Molecular Cell*, Jing et al. (2008) provide the first evidence for how