

# Transcriptional Regulator Limits Spread of Activating Chromatin Signals

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Proper gene expression is critical for the development of organisms, and its dysfunction can contribute to tumorigenesis in adult tissue. The wrapping of DNA around histones to form chromatin compacts the DNA and hinders access by many regulators of transcription. Cells use a variety of mechanisms to gain access to the embedded DNA. One important process is the post-translational modification (PTM) of histones that lowers the affinity of histones for DNA and also provides binding sites for trans-acting regulatory factors. Common modifications include histone methylation and acetylation. Methylation corresponds to both active and repressive chromatin marks, while acetylation corresponds mostly to activation of transcription.

Many important histone residues that undergo PTM and their cognate enzymes have been identified. For instance, SET domain-containing proteins exist in large protein complexes and can methylate lysine 4 of histone H3 (H3K4), e.g. Set1 methyltransferase in the COMPASS complex. Set1-mediated H3K4 methylation is involved in gene activation and is an evolutionarily conserved mechanism for transcription initiation. In humans, altered function of the MLL (Mixed Lineage Leukemia) family of SET methyltransferases contributes to an aggressive acute leukemia. Recently, a novel MLL protein named MLL5 was identified as having homology to the SET domain of MLL1, *abona fide* methyltransferase. However, MLL5 lacks critical SET domain residues that are necessary for MML1-mediated H3K4 methylation. Since MLL5 is deleted in a subset of leukemias associated with poor prognosis, uncovering its role in chromatin dynamics is of critical importance. A powerhouse Basic Sciences collaboration between the laboratories of Dr. Susan Parkhurst and Dr. Mark Groudine revealed the function of the *Drosophila* MLL5 ortholog, which is likely to be a conserved chromatin modification phenomenon.

To investigate the function of the *Drosophila* MLL5 ortholog, Dr. Hector Rincon-Arano and co-authors performed domain searches on this gene family that identified a protein with highest homology to MLL5's SET domain. The authors named this new protein UpSET. UpSET has a SET domain and a single PHD domain, the latter a protein-protein interaction motif involved in chromatin-based processes. The researchers then generated an antibody specific to UpSET and used it to stain *Drosophila* Kc cells as well as polytene chromosomes. They found that UpSET localized to the nucleus and stained ~1,000 predominately gene-rich binding sites on polytene chromosomes,

indicating a global role for UpSET in gene regulation. Similar to MLL5, UpSET's SET domain lacks residues that are expected to be necessary for catalytic activity. Indeed, recombinant UpSET did not methylate histones *in vitro*, indicating an alternative function for this elusive protein.

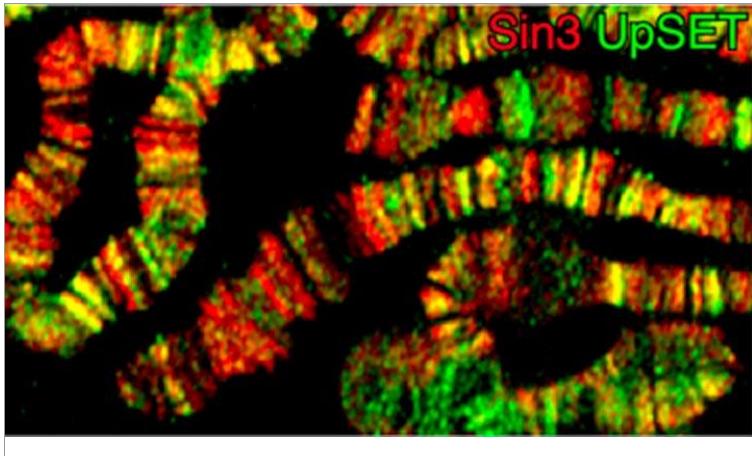
Rincon-Arano *et al.* analyzed the genome-wide association of UpSET with chromatin in Kc cells by DamID, a method of fusing Dam, a DNA methylase, to a protein of interest to mark the transcription factor's genomic binding site by adenine methylation. The researchers found that UpSET associated with ~3,500 genomic sites, many of which were in gene-rich regions. To elucidate the chromatin environment of the UpSET-bound regions, the genomic landscape occupied by UpSET was compared to known histone PTMs, transcription factor binding sites, and other data annotated in the [modENCODE](#) public database. Interestingly, UpSET was enriched at transcription start sites (TSS) and nearby promoter regions corresponding to actively transcribed chromatin, suggesting a role for UpSET in promoter regulation.

Prior knowledge of an interaction with the yeast ortholog of UpSET and the histone deacetylase Rpd3 and its accessory subunit Sin3 motivated the authors to investigate the potential interaction of UpSET with *Drosophila* Rpd3 and Sin3. Indeed, UpSET co-immunoprecipitates with Sin3 and Rpd3 from Kc cells, and immunocytochemistry of polytene chromosomes showed a high degree of UpSET and Sin3/Rpd3 co-staining (see figure). Moreover, recent genome-wide analysis showed that the HDAC machinery binds to active genes and indicated that Sin3/Rpd3 recruitment by UpSET might control promoter acetylation via HDAC recruitment. The authors discovered that promoter region acetylation increased in Kc cells depleted of UpSET as evidenced by ChIP analysis of several UpSET-regulated promoters with histone acetylation-specific antibodies. In addition, they found that UpSET is required to restrict histone acetylation and activating methylation to promoter regions since these marks spread to adjacent, normally repressed chromosomal loci in the absence of UpSET.

Finally, *upSET* mutants are female sterile and display abnormal egg chamber size and polarity, producing no mature eggs. Because signaling cascades control *Drosophila* oogenesis, the authors speculated that a particularly important pathway (Notch signaling) was hyperactive in *upSET* mutants due to improper gene activation of NOTCH and its target genes. This idea was confirmed by increased Notch protein and Notch target gene expression in *upSET* ovaries.

In summary, the results of Rincon-Arano *et al.* confirm an important methylase-independent role for the SET domain-containing protein UpSET in the fine-tuning of transcription. UpSET accomplishes this by recruiting the HDAC machinery to promoter regions. Future investigations will uncover how UpSET is recruited to chromatin to elicit transcriptional precision.

[Rincon-Arano H, Halow J, Delrow JJ, Parkhurst SM, Groudine M.](#) 2012. UpSET recruits HDAC complexes and restricts chromatin accessibility and acetylation at promoter regions. *Cell* 151:1214-1228.



*Image obtained from the manuscript*

Drosophila polytene chromosomes stained with antibodies against UpSET (green) and Sin3 (red) show significant overlap of both proteins (yellow) at many chromosomal domains. Sin3 is part of the HDAC machinery that UpSET recruits to chromatin to constrain histone acetylation to promoter regions.

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