Chronicles of the human SAGA co-activator complex

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Transcription of eukaryotic protein-coding genes involves co-activator complexes, including Transcription Factor (TF) IID and Spt-Ada-Gcn5-acetyltransferase (SAGA). A new study has determined the first high resolution cryo-EM structure of the human SAGA complex and has implications for defining SAGA function during multiple stages of eukaryotic transcription.

Eukaryotic transcription is highly regulated to ensure appropriate cell-type specific gene expression. The multi-subunit TFIID and SAGA co-activator complexes play an important role in linking epigenetic and genomic information found at enhancers and promoters to stimulate transcription of specific genes. SAGA may additionally play roles in DNA repair, mRNA export, and telomere maintenance[1,2].

Genome wide studies in yeast have shown that TFIID and SAGA have distinct and overlapping roles in transcription initiation and gene activation[3–6]. During initiation, yeast TFIID and SAGA can deposit TATA-binding protein (TBP) on gene promoters[7–9]. SAGA also regulates gene expression by acetylating lysine residues on histone tails to demarcate transcriptionally active genomic regions[10,11]. The roles of TFIID and SAGA have been less extensively interrogated in metazoan systems, and there appear to be notable differences between yeast and metazoan SAGA. For example, metazoan SAGA associates with the U2 snRNP subunits SF3B3 and SF3B5, whereas the yeast complex does not[12,13]. Conversely, metazoan SAGA does not stably interact with TBP, perhaps because a yeast TBP coordinating subunit, Spt8, is not found in metazoans[8,14,15]. Thus, between yeast and metazoans, SAGA has gained and lost specific functionalities. To help define how co-activator complexes regulate metazoan transcription, the Nogales group has pioneered efforts that have produced high resolution structures of human TFIID[7,16], and now, the structure of the ~1.4 MDa, 20 subunit, human SAGA complex[17].

SAGA is composed of four conserved functional modules. The SAGA core module is formed around a pseudo-octamer of histone fold-containing proteins. The TFIID core is similarly scaffolded, and structural similarities between TFIID and SAGA are only observed within this module. Two of the four SAGA modules have catalytic functions. The deubiquitinase (DUB) module and histone acetyl transferase (HAT) module are responsible for deubiquinating and acetylating, respectively, histones and other transcription associated proteins[10,11,18–20]. The catalytic activities of the DUB and HAT modules are important for appropriate gene expression[21]. The DUB and HAT modules are typically not observed at high resolution in cryo-EM structures of SAGA[8,22]. The fourth SAGA module is formed by the ~440kDa TRRAP subunit. Specifically, TRRAP is composed of three domains, an inactive PI3K-related pseudo kinase (ΨPIKK) domain, a

HEAT repeat domain, and a FAT domain. TRRAP and its yeast ortholog Tra1 interact with activators in a gene-specific manner and are also subunits in other co-activator complexes including TIP60 in human and NuA4 in yeast[1]. A fifth module termed the splicing module is only found in metazoan SAGA.

In this issue of Nature Structural and Molecular Biology, Herbst, Esbin *et al*., report the 2.9 Å cryo-EM structure of human SAGA[17]. The authors isolated SAGA from HeLa cells after introducing an affinity tag to core subunit SUPT7L and determined the high-resolution structure using data processing methodologies that accounted for conformational and compositional heterogeneity. Because two structures of yeast SAGA were recently reported[8,22], it is now possible to define structural similarities and differences between yeast and human SAGA (**Figure 1**).

The SAGA core module adopts a similar confirmation in the yeast and human structures. The SAGA core is connected to the TRRAP/Tra1 module by subunit SUPT20H/Spt20. Notably, deviations between the yeast and human structures are observed in the trajectory of SUPT20H/Spt20. Human SUPT20H zigzags from the core module across the TRRAP FAT domain using a linker region termed the latch. The latch region is absent in the yeast Spt20 protein. In the human structure, the latch terminates into five antiparallel beta sheets that form the SUPT20H C-terminal domain (CTD). In yeast, an N-terminal fragment of subunit Taf12 replaces the SUPT20H latch and CTD interaction with the Tra1 FAT domain.

The SUPT20H CTD forms a lid to a positively charged tunnel within TRRAP. Within this tunnel, density for inositol hexakisphosphate ($InsP_6$) is observed. The $InsP_6$ binding pocket is not well resolved in the yeast SAGA structures[8,22]. In the structure of the isolated yeast Tra1 protein, however, unassigned density is seen in the same position[23]. $InsP_6$ has been similarly observed in structures of related kinases where it is believed to serve a stabilizing role[24,25]. It remains to be determined what function $InsP_6$ plays in SAGA stability or activity.

In the human SAGA structure, repositioning of core module elements allows for the association of the splicing module. Specifically, TAF6L associates with the U2 snRNP subunit SF3B3 and serves as a bridge to the core module via an interaction with the beta-propellor domain of TAF5L. In contrast, yeast Taf5 not only interacts with Taf6 via its beta-propeller domain, but also associates with Taf6 via its N-terminus. This interaction site overlaps with the splicing module binding interface. The human TAF5L N-terminus is prevented from binding TAF6L because of an interaction with SUPT20H. Thus, additional interactions and conformational rearrangements are used to support splicing module association in human SAGA.

One of the most significant differences observed between the human and yeast SAGA structures is the relative positioning of the core module to the TRRAP module. Compared to the yeast structure, human TRRAP rotates ~75º away from the core module. This rotation results in more interactions with core module subunits. The overall binding surface area between core and TRRAP/Tra1 modules, however, is similar

between the yeast and human complexes (~3,500 Å^2). The significantly altered geometry observed between yeast and human SAGA may play an important role in the functional outcomes of the complex.

Since its discovery nearly thirty years ago, SAGA has been assigned various roles in gene activation[1]. The high-resolution structure human SAGA raises several new questions about this multi-subunit complex. First, the splicing module contains subunits that are also components of the U2 snRNP complex. Subunits SF3B3 and SF3B5 have overlapping binding interfaces with SAGA and the U2 snRNP, therefore suggesting that SF3B3 and SF3B5 are either incorporated into SAGA or the U2 snRNP, but not both simultaneously. How SF3B3 and SF3B5 contribute to SAGA function remains to be understood. Second, the large conformational changes observed between the yeast and human SAGA core and TRRAP/Tra1 modules are unlikely to be artifacts of sample preparation or data processing. Rather, these structural differences may support functional differences between the yeast and human complexes. Third, TBP is not observed to be bound to the human SAGA complex, even after the complex was incubated with high concentrations of TBP. It is unknown if additional co-factors are required for TBP binding or if, alternatively, TBP does not directly associate with human SAGA. Finally, co-activators and gene-specific transcription factors associate with SAGA. The structures of yeast and human SAGA provide a means to understand how these co-activators and transcription factors bind SAGA and may thereby potentiate gene expression. These open questions will provide exciting avenues for studying gene expression regulation in context of SAGA and other co-activator complexes.

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Figure Legend

Figure 1. Comparison of human (top) and yeast (bottom) SAGA cryo-EM structures. The cryo-EM structures of human and yeast SAGA were aligned on the TRRAP/Tra1 subunit. Corresponding subunits have the same coloring. The human TRRAP module rotates by 75º relative to yeast Tra1. Figure based on PDBs 6TBM, 6T9I, 7KTR, 7KTS, and cryo-EM maps EMD-10412, EMD-10446, EMD-23027, EMD-23028.