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Biochemistry - Viewpoint

Distinct Handoff Mechanism for TBP-TATA DNA Engagement Revealed by SAGA Structures

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Cells are able to maintain homeostasis and respond rapidly to external stimuli by carefully controlling the timing and extent of gene activation. To facilitate rapid and accurate gene expression, transcriptional coactivators must integrate interactions from transcription factors present at promoters and proximal or distal enhancers, while coordinating with the general transcription machinery at the promoter to influence transcriptional output (Figure 1). In eukaryotic cells, a fundamental first step of gene activation is the recruitment of the TATA-binding protein (TBP) to the promoter to nucleate pre-initiation complex (PIC) formation. Early biochemical and genetic studies identified a set of proteins bound to TBP, called TBP-associated factors or Tafs, that are required to activate gene expression.[1] In eukaryotes, these Tafs partition into two evolutionarily conserved multi-subunit complexes, TFIID and SAGA. While both complexes deliver TBP to promoters, each also plays distinct roles including promoter DNA-binding for TFIID while SAGA mediates enzymatic chromatin modification by its histone acetyltransferase (HAT) and deubiquitination (DUB) modules. Previously, detailed structural studies had elucidated the overall architecture and mechanism of TBP loading onto DNA by TFIID at high resolution.[2] However, an analysis of SAGA at a similar resolution and its mode of TBP-binding had remained elusive until now. Two recent landmark publications have used Cryo-EM and biochemical tools to visualize the architecture of the yeast SAGA complex. The Schultz lab has elucidated the structure of the *P. pastoris* SAGA complex and its binding to TBP, while the Cramer lab revealed the *S. cerevisiae* complex and its engagement with the ubiquitinated nucleosome.[3,4]

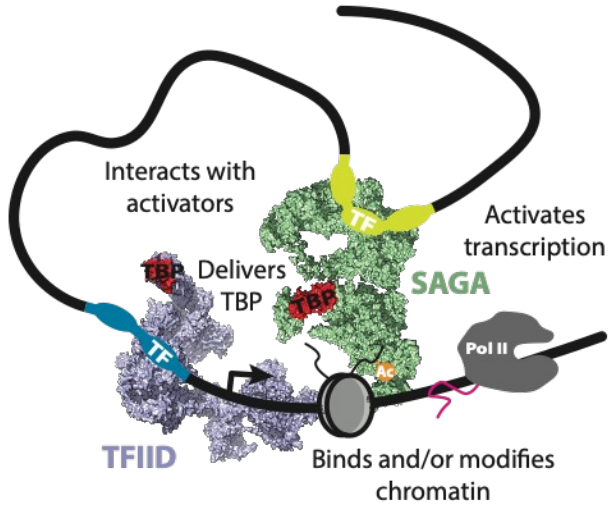

Figure 1. General transcriptional coactivators TFIID and SAGA coordinate multiple roles including activator and DNA-binding, chromatin binding and modification, and TBP delivery in order to activate gene transcription

TBP has a naturally high affinity for AT-rich sequences often present near promoters. Cells express several protein partners including TFIID and SAGA that bind TBP and prevent its association with DNA to avoid spurious transcriptional initiation. Many of these binding partners directly compete within the concave DNA-binding groove of TBP to mimic and exclude TATA DNA, as seen in TFIID's Taf1 N-terminal TAND domain and the HEAT repeats of Mot1 (BTAF1 in humans). Importantly, Papai, *et al*. were able to visualize TBP within the SAGA complex, bound at each terminus by Spt3 and Spt8.[3] As opposed to specific contacts within the concave DNA-binding surface of TBP, SAGA prevents association of TBP with DNA by steric hindrance that would cause clashes between DNA, Spt3 and part of the Tra1 subunit (Figure 2). They find that this minimal steric hindrance is enough to prevent TBP association with DNA *in vitro.* Interestingly, the presence of TFIIA is required

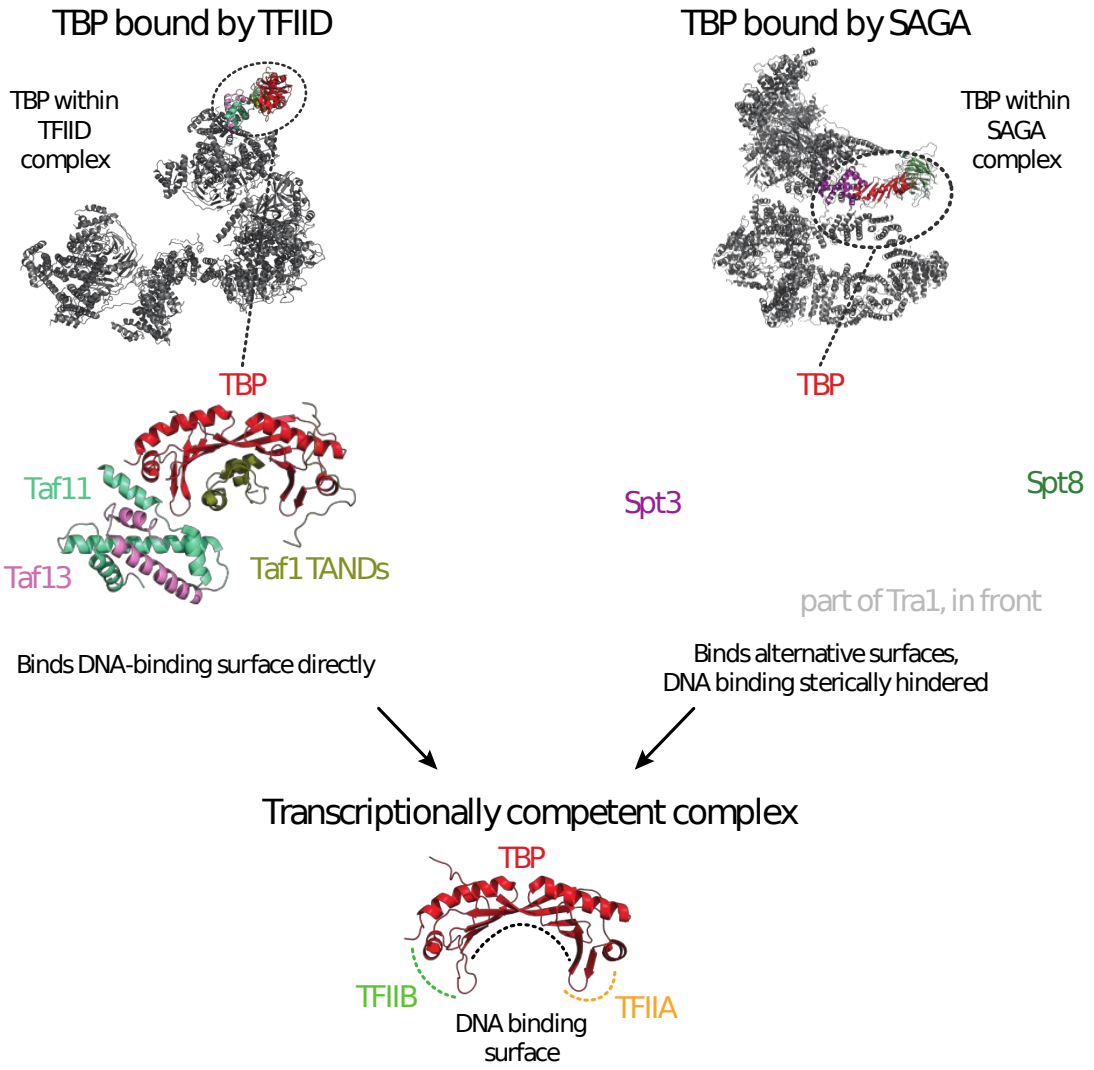

Figure 2. TBP partners employ different binding modes to prevent non-specific TBP-DNA binding and to deliver TBP to the correct sites. TFIID's Taf1 TANDs occupy the DNA binding surface directly (similarly to Mot1, not shown here), whereas SAGA binds to both termini of TBP and sterically exclude DNA. Upon DNA binding, these partners are displaced and TFIIA and TFIIB occupy TBP surfaces to nucleate PIC formation.

for release of TBP from SAGA and its subsequent binding to high-affinity TATA sequences in a competitive “handoff” model. This handoff model predicts that SAGA would briefly bring TBP in proximity to a strong TATA sequence before SAGA becomes evicted by TBP association with DNA and TFIIA followed by nucleation of a pre-initiation complex. Since handoff of TBP from SAGA appears to require both a strong TATA sequence and TFIIA, these biochemical findings support early studies which found a bias for SAGA-dominated promoters to contain consensus TATA motifs.[5] SAGA’s distinct TBP binding mode also notably leaves TBP peripheral to the main complex and the inner DNA-binding surface of TBP free, potentially allowing access to negative regulators like Mot1, which binds this concave surface. This could allow for greater competition between SAGA’s binding of TBP with other regulatory partners or facilitate higher turnover of TBP at SAGA-dominated promoters. The dynamic and coordinated delivery of TBP by the SAGA complex is therefore quite distinct from the mechanism used by TFIID. Published structural models of TFIID demonstrate downstream Tafs that stabilize binding to DNA and a dramatic rearrangement of the complex that repositions TBP at the TATA region.[2] Comparison of these apparently distinct TBP delivery mechanisms will need to be directly compared in live cells, but these new structural findings predict two fundamentally different modes of interaction with TBP deployed by TFIID versus SAGA at the promoter.

In addition to TBP delivery, these new studies reveal dynamic regulation of SAGA upon nucleosome binding. In Wang, *et al*. the authors find that SAGA undergoes large structural rearrangements after binding to a ubiquitinated nucleosome, with the HAT and DUB module conformation becoming undefined (potentially mobile) and essentially invisible in Cryo-EM structures.[4] As a complex, SAGA must somehow balance this flexible architecture with precise functions in TBP binding, histone tail recognition, and enzymatic activity. Upon binding of the DUB module to the face of the downstream nucleosome, the authors postulate that SAGA would be well-positioned to bridge activators at the yeast upstream activating sequence (UAS) to the promoter, anchoring the activator-binding subunit Tra1 upstream of the UAS, thus leaving TBP well-positioned over the TATA region. In contrast to the proximal and more stringently regulated UAS-promoter distances in yeast, mammalian enhancers and their corresponding promoters often span large genomic distances. In mammals, where distal regulatory elements are often bound by TFs tens of kilobases away from the promoter, it is unclear how SAGA would bridge or maintain contacts between enhancers and promoters. This may point to a more dynamic model in humans where SAGA must somehow balance activity between enhancers and promoters over large distances, perhaps by some hit-and-run dynamic mechanism or even where SAGA may influence the 3D genome organization by flexibly tethering enhancers and promoters.

Despite decades of study of TFIID and Taf-containing complexes, understanding the unique regulatory roles, genomic targets, and structure of SAGA has remained elusive. Two new studies using biochemical and high-

resolution structural data, at least, partially unveil the interwoven architecture of the Taf-containing SAGA complex. A growing number of studies, including these two, solidify the emerging view that most biological processes likely rely on much more dynamic and transient, as opposed to static, “lock-and-key” protein interactions. These studies reveal a novel mechanism of TBP handoff by SAGA and dynamic nucleosome binding with implications for initiating transcription. While there are many questions yet to resolve, understanding the architecture and structure of the SAGA complex may be a launchpad to more fully appreciate the dynamic spatiotemporal regulatory mechanisms of gene activation.

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Notes

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