hydrophobic interactions. Alternatively, the progesterone might be bound to an external substrate-recognition or effector site. Progesterone interacts directly with Asp214; mutation of Leu211 and Asp214 to increase side-chain size eliminates the homotropic cooperativity of progesterone metabolism and decreases heterotropic cooperativity of α-napthoflavone with progesterone and testosterone [21]. These changes in cooperativity are consistent with a role for Leu211 and Asp214 as part of an effector site.

Concluding remarks and future perspectives

The determination of CYP3A4 structures is a significant step towards understanding the binding of ligands to this important drug-metabolizing enzyme. Additional structures describing the binding of substrates within the active-site cavity and with multiple substrates that demonstrate non-Michaelis–Menton kinetics will be required to gain a further understanding of the regiospecific metabolism and complex cooperativity exhibited by CYP3A4.

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SAGA unveiled

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Transcriptional regulation in eukaryotes is intimately coupled to chromatin dynamics. The SAGA (Spt–Ada–Gcn5) histone acetyltransferase (HAT) complex of *Saccharomyces cerevisiae* is a multi-subunit co-factor for RNA polymerase II transcription. However, not all gene activation events require its intrinsic HAT activity. In addition, SAGA subunits can also restrict gene transcription. The recently published structural model from the laboratories of Fred Winston and Patrick Schultz of the SAGA complex provides a framework to

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rationalize these findings and to direct further investigation of this crucial transcriptional co-factor.

Efficient regulation of RNA polymerase II (pol II)-mediated transcription in eukaryotes depends on the interplay of many types of regulatory proteins of transcription. Large multi-subunit complexes capable of mobilizing or modifying chromatin proteins have been identified as co-factors for regulation by gene-specific transcription factors, which bind to their cognate sites in enhancer and promoter-distal DNA elements.

The SAGA (Spt–Ada–Gcn5) histone acetyltransferase (HAT) complex of *Saccharomyces cerevisiae* represents a paradigm for multi-subunit transcriptional co-factors. SAGA was first identified biochemically as a HAT for free and nucleosomal histone H3 [1]. The 1.8-MDa SAGA complex comprises products of distinct classes of genes: (i) the Ada proteins (Ada1, Ada2, Ada3, Gcn5 and Ada5), which have been isolated in a genetic screen for interactors of the gene-specific activator Gcn4 and the activation domain of herpes simplex virus VP16; (ii) the TATA-binding protein (TBP)-related set of Spt proteins (Spt3, Spt7, Spt8 and Spt20), initially identified as suppressors of defects in transcription initiation caused by insertions of the Ty transposable element; (iii) a subset of TBP-associated factors (TAFs; TAF5, TAF6, TAF9, TAF10 and TAF12); and (iv) the product of the essential *TRA1* gene (Locus tag: YHR099W) [2]. Gcn5 is the catalytic HAT subunit [1], and is regulated by Ada2 and Ada3 [3,4]. TAF12, Spt7, Spt20 and Ada1 are required for SAGA integrity [4,5]. A mutation in Spt3 was isolated as an allele-specific suppressor of a TBP mutant [6]. Moreover, intact SAGA interacts with TBP; this requires the Spt8 and Ada3 subunits, but is independent of Spt3 [4]. Tra1, an ataxia telangiectasia mutated (ATM)-related protein, is a target for several activators including Gcn4, VP16 and Gal4 [7,8]. In addition to these subunits, novel SAGA components have recently been described: Ubp8, a ubiquitin-specific protease; Sgf11, required for anchoring of Ubp8; Sgf29 and Sgf73, with unknown function; and Sus1, which is also part of the nuclear pore-associated mRNA export machinery [9–11]. In SAGA, the existence of three putative histone-fold domain (HFD)-containing heterodimer pairs has been suggested: TAF6–TAF9, TAF10–Spt7 and TAF12–Ada1 [12]. A variant of the SAGA complex, named SALSA or SLIK, which lacks Spt8 and contains a truncated form of Spt7, has been isolated, but its functional role is unclear [13,14].

Orthologous Gcn5 HAT complexes from metazoans

Several orthologous Gcn5 HAT complexes have been characterized from *Drosophila* (see Ref. [15] and refs therein) and human cells, such as the TBP-free TAF-containing complex (TFTC), the p300- and CBP-associated factor (PCAF) or GCN5 complexes and the STAGA complexes, all of which contain Gcn5, Ada proteins, Spts, TAFs and TAF-like proteins, in addition to the Tra1 orthologue, TRRAP (transformation/transcription domain-associated protein) [2]. Recently, ataxin-7 – encoded by the spinocerebellar ataxia type 7 (SCA7) gene (the human orthologue of *Sgf73*) – was shown to be a TFTC subunit [16]. Numerous studies have shown that SAGA complexes and TFTC-like complexes are recruited to chromatin templates by activators of acetylate histones and, thus, regulate transcription [2,17–19].

Three-dimensional structure of SAGA

A major breakthrough in the understanding of SAGA is represented by its recent 3D structure elucidation using electron microscopy (EM) methods by the Schultz (http://www-igbmc.u-strasbg.fr/Departments/Dep_VI/Dep_VIF/Dep_VIF1.html) and Winston (http://genetics.med.harvard.edu/%7Ewinston) laboratories [20]. In the study, negatively stained SAGA particles were viewed using EM from different angles. Image reconstruction yielded a 3D model at ~30-Å resolution, which revealed that SAGA consists of five modular domains with diameters of 70–100 Å (Figure 1). An overlay of the structures of yeast SAGA and human TFTC indicates, in good agreement with their similar subunit composition, a high degree of structural conservation in size and shape [20,21].

Mapping of the subunits of SAGA using immuno-EM methods has revealed specialized functions of the distinct domains of SAGA (Figure 1 and Table 1). Domain I contains Tra1, which seems to represent the activator interaction surface. Domains II, III, and IV contain several HFD-containing TAFs and TAF5. Moreover, TAF5 might have an architectural role in SAGA, similar to its role in TFIID. In domain III, the two bromodomain-containing subunits, Gcn5 and Spt7, were also detected. Results from biochemical and genetic interaction studies suggest that they would co-localize with Ada2 and Ada3 (Figure 1 and Table 1), indicating that domain III is not only a central architectural domain, but also harbours HAT activity. Domain V – the structure of which seems to be more flexible – contains Spt3, Spt20 and probably Spt8; together, these define the TBP-interacting module (Figure 1 and Table 1). Domain V is variable in the 3D structure, which is

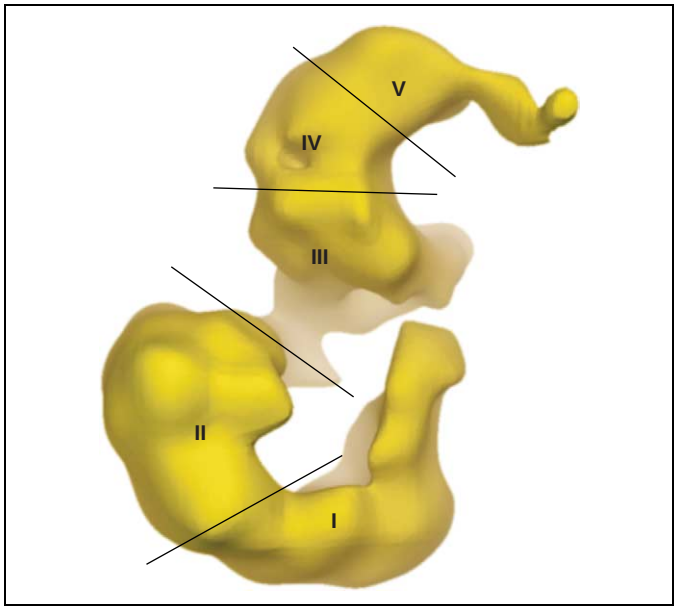

Figure 1. Three-dimensional surface representation of the SAGA model containing the flexible domain. The complex is ~18×28 nm in size and comprises five domains [20]. The five domains, which contain distinct sets of SAGA subunits, are indicated and separated by bars.

Table 1. The location of the different subunits within the distinct domains of SAGA

Domain	Examined[a]	Hypothesized[b]
I	Tra1*	–
II	TAF5, TAF6	TAF9, TAF10
III	GCN5, TAF10, SPT7*, TAF5, Ada1	Ada2, Ada3, TAF12
IV	Ada1, TAF6	TAF9, TAF12
V	Spt3*, Spt20	Spt8

[a]'Examined' factors were labelled with either polyclonal antibodies raised against the native proteins or antibodies raised against a tagged version of a given subunit (asterisks).
[b]Subunits were suggested to be in the indicated domains based only on previous biochemical or genetic data.

in agreement with the finding that Spt8 is not present in all SAGA complexes and, thus, might associate dynamically with SAGA as a regulatory subunit [13,14].

Structure–function relationship between SAGA and TFIID

Transcription factor IID (TFIID) is a multi-subunit complex involved in the initiation of pol II transcription. TFIID contains TBP, specific TAFs and TAF subunits that are also present in SAGA; one of its specific subunits, TAF1, harbours HAT activity [2]. SAGA and TFIID make overlapping contributions to the expression of pol II-transcribed genes. TFIID function seems to predominate the transcription of ~90% of the yeast genome, whereas SAGA might have an important role in the transcription of ~10% of the genes, most of which seem to be stress-induced [22]. At these promoters, SAGA is required for recruitment of the basal transcription machinery and probably for promoter delivery of TBP [17,23]. Importantly, whereas TFIID binds to the core promoter, SAGA interacts with DNA via upstream activating sequences (UASs) [23,24]. Human TFTC directs pre-initiation complex assembly (PIC) *in vitro* on naked DNA templates irrespective of a canonical TATA element and in the absence of detectable amounts of TBP [25]. Thus, it seems that both the yeast and the human complexes directly participate in PIC assembly. Although the exact mechanism remains to be determined, comparison of the low-resolution structures of SAGA and yeast TFIID reveal important similarities and differences, which can be relevant for their function in transcription and histone acetylation [20].

The spatial distribution of TAF5, TAF6 and TAF10 in SAGA and TFIID is similar, and this was used to align these two complexes [20,26]. The alignment suggests that the 4-nm wide groove of TFIID, which could be involved in DNA binding, is similar to the cleft formed by domains II, III, and IV of SAGA. The location of TAF5, together with the HFD-containing proteins in both complexes, indicates that, similar to TFIID, the HFD-heterodimer pairs within SAGA are connected by the WD40 repeat-containing TAF5 protein to form the structural backbone of the complex. In TFIID, all HFD-containing TAF pairs can be found in two different domains [26]. In SAGA, TAF6–TAF9 and Ada1–TAF12 are the only HFD-containing pairs that have been localized twice and in different domains (Figure 1 and Table 1). The TAF10–Spt7 HFD heterodimer could be localized with certainty only in domain III of SAGA; it might also be present in domain II, but internal symmetry of some SAGA views makes this uncertain [20] and, thus, Spt7 seems to be restricted to domain II. However, if the uncertain immuno-labelling of TAF10 in domain II indeed reflects the second copy of TAF10, this would imply that TAF10 has a second, as yet unknown, interaction partner in domain II of SAGA.

Alignment of the SAGA and TFIID structures indicates that their respective HATs are positioned at different locations: the HAT domain of TAF1 would be at the equivalent of domain II of SAGA, whereas GCN5 is in domain III [20,27]. Despite common subunits, structural similarity and functional relationships, the location of HAT activity and the bromodomain-containing factors in the respective complexes suggests that SAGA and TFIID will interact with chromatin in a different way. Another interesting comparison between the two complexes is how they interact with TBP. TBP is a stable component of TFIID and localizes to the thin connecting bridge between lobe A and C (see Ref. [27] and refs therein), which is equivalent to the connection between domain II and III in SAGA. By contrast, intact SAGA does not contain, or contains only small amounts of, TBP [9]. The Spt3–SPT20–Spt8 module, which seems to control TBP interactions with promoters, is in domain V of SAGA, which is absent from TFIID. Interestingly, Spt3 contains two HFDs that might form an intramolecular TAF11–TAF13-like pair in SAGA [12], and Spt8 is a WD40 repeat-containing protein [28]. In SAGA, Spt8-TBP and Spt3-TBP interactions seem to be inhibitory in the uninduced state of certain promoters, whereas TBP–Spt8–TFIIA interactions seem to cooperate in PIC formation during activated transcription (see Ref. [29] and refs therein). This indicates that gene regulation by SAGA and TFIID complexes might be mechanistically different, a view supported by the obtained structural data. The different locations of TBP in SAGA and TFIID also provide an explanation for why these complexes can only be cross-linked to either the UAS or to the TATA element, respectively. The association of SAGA to a UAS element could direct the Spt3–Spt20–Spt8 module to position the TBP–TFIIA complex at a downstream position on the core promoter, which might explain why SAGA complexes support both TATA and TATA-less transcription.

Concluding remarks

These new data on the structure of SAGA describe its relationship with the TFIID complex and show that the shared TAF subunits form the backbone of these multi-subunit complexes that are crucial for eukaryotic transcription. The SAGA structure itself, however, reveals a new piece in the puzzle of transcription, but the game is not over yet. Some challenges remain: (i) how do SAGA, protein complexes of the SWI/SNF family (which remodel nucleosome structure in an ATP-dependent manner), mediator, TFIID, TBP, Mot1 and other 'pieces' fit together in time and space? (ii) How can SAGA act to restrict transcription at certain genes? (iii) What determines SAGA- versus TFIID-dependence of a given promoter? And (iv) how are SAGA and TFIID complexes distributed over the genome under different conditions?

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