Structure of the transcription coactivator SAGA

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Gene transcription by RNA polymerase II is regulated by activator proteins that recruit the coactivator complexes SAGA (Spt–Ada–Gcn5–acetyltransferase)2–4 and transcription factor IID (TFIID)2–4. SAGA is required for all regulated transcription5 and is conserved among eukaryotes6. SAGA contains four modules7–9; the activator-binding Tra1 module, the core module, the histone acetyltransferase (HAT) module and the histone deubiquitination (DUB) module. Previous studies provided partial structures10–14, but the structure of the central core module is unknown. Here we present the cryo-electron microscopy structure of SAGA from the yeast Saccharomyces cerevisiae and resolve the core module at 3.3 Å resolution. The core module consists of subunits Taf5, Sgf73 and Spt20, and a histone octamer-like fold. The octamer-like fold comprises the heterodimers Taf6–Taf9, Taf10–Spt7 and Taf12–Adal, and two histone-fold domains in Spt3. Spt3 and the adjacent subunit Spt8 interact with the TATA box-binding protein (TBP)2,15–17. The octamer-like fold and its TBP-interacting region are similar in TFIID, whereas Taf5 and the Taf6 HEAT domain adopt distinct conformations. Taf12 and Spt20 form flexible connections to the Tra1 module, whereas Sgf73 tethers the DUB module. Binding of a nucleosome to SAGA displaces the HAT and DUB modules from the core-module surface, allowing the DUB module to bind one face of an ubiquitinated nucleosome.

The structure confirms the overall topology of SAGA with four flexibly connected modules15–18 and reveals the intricate subunit architecture of the coactivator complex (Fig. 1). The SAGA structure contains only one copy of each subunit, in contrast to TFIID, which contains two copies of several subunits14. The SAGA core module occupies a central position and comprises the subunits Taf5, Taf6, Taf9, Taf10, Taf12, Spt3, Spt7, Spt20 and Adal. The TBP-interacting subunit Spt8 is flexibly connected to the core module, as are the HAT and DUB modules (Fig. 1a). These three functional SAGA regions are lined up on one side of the complex that is predicted to face promoter DNA (Fig. 1a).

The core module contains a histone octamer-like fold and an adjacent subodule formed by subunits Taf5, Taf6 and Spt20 (Fig. 2). The octamer-like fold comprises three pairs of subunits that each contribute one histone fold, namely Taf6–Taf9, Taf10–Spt7 and Taf12–Adal, and Spt3, which contributes two histone folds. The presence of an octamer-like fold explains early observations of histone-like subunit pairs in SAGA15,16. In contrast to a canonical histone octamer, which shows twofold symmetry, the SAGA octamer-like fold is fully asymmetric (Extended Data Fig. 3a).

Taf5 connects the octamer-like fold to the remainder of the core module and is thus important for core-module architecture (Fig. 2b).

SAGA contains 19 subunits, distributed over four modules8,24. The Tra1 module binds activators25,26, the core module recruits TBP19, the HAT module contains the histone H3 acetyltransferase Gcn5 and the DUB module comprises a histone H2B deubiquitinase22,23. To determine the structure of SAGA, we purified the endogenous complex from S. cerevisiae using a strain with a C-terminal TAP-tag on subunit Spt20 (Methods). Purified SAGA contained all 19 subunits in apparently stoichiometric amounts (Extended Data Fig. 1a); we analysed this complex using cryo-electron microscopy (cryo-EM) and protein cross-linking analysis (Methods). We obtained a reconstruction of SAGA at an overall resolution of 3.9 Å (Extended Data Fig. 1, Supplementary Video 1).

The two large SAGA modules—the Tra1 and the core module—were resolved at 3.4 Å and 3.3 Å resolution, respectively (Extended Data Figs. 1d, e, 2a). We fitted the Tra1 structure24, built the core module and the protein regions connecting the two modules and refined the structure in real space (Extended Data Tables 1, 2, Extended Data Fig. 2b). The HAT and DUB modules were more flexible and were resolved at 9 Å and 12 Å resolution, respectively. The structure of the DUB module25 could be fitted, but density for the HAT module could not be interpreted (Supplementary Video 1). Our protein–protein cross-linking analysis and previous cross-linking data27 validated our modelling and assigned subunit Spt8 to a remaining density located between the core and Tra1 modules (Supplementary Table 1, Extended Data Fig. 2c, d).
contains a SEP (shp1–eyc–p47) domain (Fig. 2c). Spt20 also contains an extended loop that forms a wedge between the two Taf5 domains, thereby stabilizing them in a defined orientation (Fig. 2b, Extended Data Fig. 3b). The Lis1 homology motif (LisH) helices of Taf5 interact with the SEP domain of Spt20 (Extended Data Fig. 3c). Taf6 contributes one β-strand to the Taf5 propeller, suggesting that Taf5 and Taf6 form an obligate heterodimer (Fig. 2c). The Taf5 propeller and the Taf6 HEAT region both interact with a stretch of Spt7 that extends from the octamer-like fold and continues into a bromodomain that is mobile.

SAGA has long been known to bind TBP15–17, implying a role in recruiting TBP to promoters. According to cross-linking7 and genetic data2, SAGA interacts with TBP via the Spt3 and Spt8 subunits. Spt3 and Spt8 occupy adjacent locations at the edge of the octamer-like fold (Figs. 1, 3a). SAGA and TFIID share the subunits Taf5, Taf6, Taf9, Taf10 and Taf1218. TFIID consists of three lobes—A, B and C—and has been structurally defined3,4. Lobe A contains an octamer-like fold that resembles the fold observed in SAGA. However, Spt7 and Ada1 are replaced by TFIID subunits Taf3 and Taf4, respectively, and the two histone-fold domains of Spt3 are replaced by the histone fold pair Taf11–Taf13 in TFIID (Fig. 3b). Despite these differences, the octamer-like folds in SAGA and TFIID bind TBP at the same relative position (Fig. 3a, b).

We generated a model of the SAGA–TBP complex by superposing the TBP-containing TFIID structure lobe A onto the SAGA core structure (Extended Data Fig. 3d). The model is consistent with TBP bridging between SAGA subunits Spt3 and Spt8. In TFIID, the TFIID-specific subunit Taf1 also contributes to TBP binding, and this may explain an apparently higher affinity of TFIID for TBP compared with SAGA2. TFIID lobe B contains a hexamer of histone-fold domains that lacks the Taf11–Taf13 pair and does not bind TBP, but otherwise resembles its counterpart in SAGA (Fig. 3c).

Structural comparisons also show how SAGA and TFIID form distinct structures despite sharing five subunits (Fig. 3). The shared subunits Taf5 and Taf6 have different structures in the two complexes. In TFIID, the Taf5 N-terminal domain docks to the octamer-like fold and is stabilized by the TFIID-specific subunit Taf4. In SAGA, Taf4 is absent and subunit Ada1 occupies its position. The Taf5 N-terminal domain occupies a position that is distant from the octamer-like fold, is stabilized by the SAGA-specific subunit Spt20 and contacts the Taf6 HEAT repeat domain. Underlines indicate regions included in the structure. B, Ribbon model showing subunit arrangement and interactions. View and colour code as in Fig. 1. C, The Taf5 WD40 propeller domain interacts with six other SAGA subunits.
Fig. 3 | Comparison of the SAGA core module with TFIID. a, SAGA core module with subunits that are shared with TFIID in colour. A magenta dot depicts lysine residue K190 of Spt3 that was cross-linked to TBP\(^{18}\) and is located in the loop between the two histone folds of Spt3 (dashed magenta line). b, Comparison with TBP-bound TFIID lobe A\(^{3}\) shows that TBP binds to the same relative position with respect to the histone-like fold in SAGA and TFIID. The histone-like folds are similar but differ in their subunit composition. c, Comparison with TFIID lobe B\(^{3}\) reveals different structures of Taf5 and Taf6 that are due to complex-specific subunits.

Fig. 4 | Nucleosome binding induces changes in SAGA. a, Structure of DUB–nucleosome complex within SAGA. b, Model showing changes in SAGA module orientation on nucleosome and promoter binding.
counterparts of all yeast SAGA subunits except Spt8\(^{26}\) (Extended Data Table 3). Thus, our yeast SAGA structure is a good model for yeast SLIK and human SAGA. In conclusion, the structure of SAGA integrates available data, reveals differences to TFIIID and provides a framework for studying the mechanisms used by this multifunctional coactivator to regulate transcription.

Online content

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Methods

Purification of endogenous SAGA

*S. cerevisiae* strain CB101 (MATa pep4::HIS3, prb1::LEU2, prc1::HIS3, can1, ade2, trpl, ura3, his3, leu2-3,112) with a C-terminal TAP tag at Spt20 was grown in a 200-l fermenter (INFORS-HT) with 100 l YPD medium overnight and collected at OD600 = 5. Cell pellets were resuspended in lysis buffer (30 mM HEPES pH 7.5, 300 mM NaCl, 1.5 mM MgCl2, 0.05% NP-40, 1 mM DTT, 0.254 μg ml−1 leupeptin, 1.37 μg ml−1 pepstatin A, 0.17 mg ml−1 PMSF, 0.33 mg ml−1 benzamidine) and frozen in liquid nitrogen. Frozen yeast cell beads were milled to powder using a cryogenic grinder (Spx sample prep 6875D). The lysed yeast powder was thawed and mixed with half the volume of lysis buffer. Lysates were cleared by centrifugation (4,000g, 4 °C, 20 min and 235,000g, 4 °C, 60 min). The purification was performed as described30, with several modifications. In brief, the supernatant was incubated with IgG Sepharose-6 Fast Flow resin (GE Healthcare) at 4 °C for 3 h, the resin was washed with 5 column volumes of lysis buffer followed by 5 column volumes of TEV cleavage buffer (30 mM HEPES pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 0.05% NP40, 1 mM DTT and 0.5 mM EDTA) and then resuspended in 5 ml of the TEV cleavage buffer. TEV cleavage was performed by incubating with His6-TEV protease for 16 h at 4 °C. The eluate was loaded onto a 1 ml HiTrap Q column (GE Healthcare) and eluted with a gradient using as high salt buffer 30 mM HEPES pH 7.5, 1.5 M NaCl, 1.5 mM MgCl2, 1 mM DTT. Peak fractions were concentrated to approximately 1 mg ml−1.

Preparation of modified nucleosomes

To generate the K120-ubiquitylated histone H2B, we introduced a lysine-to-cysteine mutation (K120C) into the *Xenopus* H2B sequence and a glycine-to-cysteine mutation (G76C) to ubiquitin by site-directed mutagenesis. The dichloroacetonitrile cross-link was formed between ubiquitin and H2B-K120 as described30, with minor changes. In brief, 100 μM H2B-K120 and 100 μM His6-Ub(G76C) proteins were incubated at 50 °C in reaction buffer (50 mM borate pH 8.1, 1 mM tris(2-carboxyethyl) phosphine (TCEP)) for 1 h to reduce cysteines, and were then cooled on ice for 1 h. Dimethyl formamide (DMF) dissolved in dichloroacetonitrile was added to the solution to a final concentration of 100 μM and incubated on ice for 1 h. The reaction was quenched with 50 mM β-mercaptoethanol, frozen and lyophilized. The resulting product mixture was resuspended in Ni-UB buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 6 μM urea, 2 mM β-mercaptoethanol, 20 mM imidazole) and applied to a HiTrap HP 5 ml column (GE Healthcare). The bound proteins were eluted with Ni buffer supplemented with 150 mM imidazole, and dialysed into TEV cleavage buffer. After TEV cleavage for 16 h at 4 °C, the product was dialysed into Ni-UB buffer and reapplied to a HiTrap HP 5 ml column to remove uncleaved products. The flow-through from the column was applied to a HiTrap SP 5 ml column (GE Healthcare) and eluted with a gradient of Ni-UB with 1M NaCl. Peak fractions were pooled and dialysed to water containing 5 mM β-mercaptoethanol, frozen and lyophilized.

H3K4me3 binding by the Sgf29 Tudor domain is required for chromatin targeting and histone H3 acetylation of SAGA32. To generate the K4-trimethylated histone H3 variant, a single lysine-to-cysteine mutation (K4C) was introduced into the H3 sequence by site-directed mutagenesis. Cysteine-engineered histone H3 K4C protein was alkylated as described30. In brief, purified protein was reduced with DTT before addition of a 50-fold molar excess of trimethylammonium bromide (Sigma I7196-25G). The reaction mixture was incubated for 4 h at 50 °C before quenching with 5 mM β-mercaptoethanol. The modified protein was desalted using a PD-10 desalting column (GE Healthcare) pre-equilibrated in water supplemented with 2 mM β-mercaptoethanol and lyophilized. Successful alkylation was confirmed by MALDI-TOF mass spectrometry. The Widom 60114S bp DNA was purified as described from the pUC19 8 × 145 bp 601-sequence plasmid using the restriction enzyme EcoRV to digest the DNA into fragments34. Nucleosomes were reconstituted with modified histones and the Widom 601 DNA as described34.

Cryo-EM sample preparation

Purified SAGA (or SAGA mixed with the modified nucleosome at a molar ratio of 1:2) was incubated with 3 mM BS3 for 1 h on ice, and quenched for 10 min using 10 mM Tris-HCl pH 7.5, 2 mM lysine and 8 mM aspartate. Quenched samples were applied to a 15–40% sucrose gradient in dialysis buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 1 mM TCEP, 2% glycerol), and ultracentrifuged at 32,000 rpm (SW60 rotor) for 16 h at 4 °C. Gradients were fractionated in 200 μl and analysed with native PAGE. The gels were stained with Syber Gold (Invitrogen) and Coomasie brilliant blue. Peak fractions containing SAGA or the SAGA–nucleosome complex were dialysed overnight, concentrated to approximately 0.2 mg ml−1 and used for grid preparation. Two microliters of sample was applied to glow-discharged UltrAuFoil 2/2 grids (Quantifoil) on each side of the grid. After incubation for 10 s, the sample was blotted for 4 s and vitrified by plunging into liquid ethane using a Vitrobot Mark IV (FEI Company) operated at 4 °C and 100% humidity.

Cryo-EM data collection and image processing

Cryo-EM data of the SAGA and SAGA–NCP were acquired on a FEI Titan Krios transmission electron microscope operated at 300 keV, equipped with a k2 summit direct detector and a GIF quantum energy filter (Gatan). Automated data acquisition was carried out using EPU software (FEI) at a nominal magnification of 130,000× or 105,000×, resulting in calibrated pixel sizes of −1.05 Å and −1.35 Å for SAGA and the SAGA–nucleosome complex, respectively. Movies of 40 frames were collected in counting mode over 9 s with a defocus range of 1.25–2.75 μm. The dose rate was 4.7 e− Å−2 s−1 resulting in 1.06 e− Å−2 per frame for SAGA, and 4.9 e− Å−2 s−1 resulting in 1.10 e− Å−2 per frame for the SAGA–nucleosome complex, respectively. A total of 4,697 and 4,866 movies were collected for SAGA and the SAGA–nucleosome complex, respectively. Movie stacks were motion-corrected, CTF-estimated and dose-weighted using Warp31.

Particles of the SAGA data were auto-picked by Warp, yielding 250,368 particle images. Image processing was performed with RELION 3.0.5.37. Particles were extracted using a box size of 4002 pixels, and normalized. Reference-free 2D classification was performed to screen for good particles in the dataset. An ab initio model generated from cryoSPARC was used as an initial reference for subsequent 3D classification. All classes containing intact SAGA density were combined (107,759 particles) and used for a global 3D refinement resulting in a map at 4.7 Å resolution. To improve the map for the core module of SAGA, focused 3D classification without image alignment was performed using a mask around the core module. The class that showed the best density for the core module was subjected to another round of 3D refinement resulting in an overall resolution of 4.1 Å. Focused refinement further improved the resolution to 3.4 Å and 3.3 Å for Tra1 and the core module, respectively. Post-processing of refined reconstructions was performed using automatic B-factor determination in RELION and reported resolutions are based on the gold-standard Fourier shell correlation (FSC) 0.143 criterion (B-factors of −107 Å2 and −91 Å2 for the Tra1 and the core module, respectively). Local resolution estimates were obtained using the built-in local resolution estimation tool of RELION using the estimated B-factors.

For the SAGA–nucleosome complex sample, 579,759 particles were auto-picked by Warp. As the DUB–nucleosome and the remaining parts of SAGA were not present together during the classification steps, particles of SAGA in the nucleosome-bound state and the DUB–nucleosome were not present together during the classification steps, particles of SAGA in the nucleosome-bound state and the DUB–nucleosome were processed separately. Otherwise, the processing procedure was the same as that for SAGA. However, focused 3D classification without alignment did not yield good core-module particles from the SAGA–nucleosome dataset. A reconstruction at 6.1 Å overall resolution was obtained from 86,910 particles of SAGA in the nucleosome-bound state.
Focused refinement further improved the resolution to 4.2 Å for the Tra1 lobe. For the DUB–nucleosome complex, a reconstruction at 3.7 Å overall resolution was obtained from 113,856 particles. Post-processing of the refined reconstructions was performed using automated B-factor determination in RELION and reported resolutions are based on the gold-standard FSC 0.143 criterion (B-factors of −149 Å² and −115 Å² for the Tra1 lobe and the DUB–nucleosome, respectively). Local resolution estimates were obtained using the built-in local resolution estimation tool of RELION using the previously estimated B-factors.

Cross-linking and mass spectrometry
Samples for cross-linking mass spectrometry were purified in the same way as those for cryo-EM. Cross-linked samples were filtered with 1% FDR and plotted using xVis40.

Model building
The structure of the core module was built by first placing the known structure of the Taf5–Taf6–Taf9 trimer (PDB ID: 6F3T) into the density by rigid-body fitting in Chimera. Adjustments were made to the protein sequence in Coot44; insertions and deletions were manually built according to the density. The histone-fold domains of Taf10, Sp7, Taf2, Ada1 and Sp3 and extensions from them were manually built. The structure of the Taf5 NTD (PDB ID: 2J49) and Taf6 HEAT domain (PDB ID: 4ATG) were placed into the density and adjusted in Coot. The remaining parts were built manually. Secondary structure predictions from PSIPRED were used to assist de novo modelling. α-helices were generated using Coot and manually fitted into the density. Linkers between the helices were modelled where clear density was visible. Cross-linking restraints and densities from bulky residues such as Lys, Arg, Phe, Tyr and Trp were used to guide modelling. The SEP domain of Sp20 shares structural homology with human p47 (PDB: 1S56), and this structure guided Sp20 modelling. The structure of the Tra1 module was built by placing the structure of Tra1 (PDB ID: 5OJS) into the density by rigid-body fitting in Chimera, and the TIRs of Taf12 and Sp20 were manually built in Coot based on the density and cross-linking restraints. The DUB–nucleosome structure (PDB ID: 4ZUX) was placed into the corresponding densities by rigid-body-fitting the DUB module and nucleosome in Chimera. All models were subjected to alternating manual adjustment and real-space refinement using Coot and PHENIX45, resulting in good stereochemistry as assessed by Molprobity46. Figures were generated in PyMOL (Schrödinger, v.2.2.2) and UCSF Chimera (v.1.13).

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
The electron density reconstructions and models of the complete SAGA complex, the Tra1 module, the core module, the DUB module–nucleosome complex and the nucleosome-bound state of SAGA were deposited with the Electron Microscopy Data Bank (accession codes EMD-10412, EMD-10413, EMD-10414, EMD-10415 and EMD-10416 respectively) and with the Protein Data Bank (accession codes 6T91, 6T9J, 6T9K, and 6T9L). All the other relevant data are included in the Supplementary Information or are available from the authors upon request.

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Supplementary Information

Model building
The structure of the core module was built by first placing the known structure of the Taf5–Taf6–Taf9 trimer (PDB ID: 6F3T) into the density
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**Author contributions** H.W. carried out all experiments and data analysis except mass spectrometry analysis. C.D. assisted with cryo-EM data collection. A.C.M.C. contributed to developing the purification protocol and assisted with model building. A.S. and H.U. carried out mass spectrometry analysis. P.C. supervised research. H.W. and P.C. interpreted the data and wrote the manuscript, with input from all authors.

**Competing interests** The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Cryo-EM structure determination and analysis of SAGA. Related to data shown in Fig. 1. a, Purification of endogenous SAGA from S. cerevisiae. SDS–PAGE of peak fraction used for cryo-EM grid preparation. Identity of the bands was confirmed by mass spectrometry. For gel source data, see Supplementary Fig. 1. b, Exemplary cryo-EM micrograph of data collection. The micrograph is shown before (left) and after (right) denoising using Warp. c, The 2D class averages. d, Sorting and classification tree used to reconstruct SAGA. e, FSC between half maps of the final reconstructions of the complete SAGA complex and the SAGA modules Tra1 and core. Resolutions for the gold-standard FSC 0.143 criterion are listed. f, Angular distribution plot for all particles in the final reconstructions of the SAGA core (top) and Tra1 (bottom) modules. Colour shading from blue to yellow correlates with the number of particles at a specific orientation as indicated.
Extended Data Fig. 2 | Quality of the SAGA structure. Relates to data in Figs. 1, 2. 

**a**, SAGA reconstruction coloured according to local resolution. Model–map 
FSC curves calculated between the refined atomic models and maps are shown 
below. **b**, Electron density (grey transparent surface) for various SAGA regions 
as indicated. **c**, Overview of the cross-linking data. Circular plot of high- 
confidence lysine–lysine intersubunit (green) and intrasubunit (purple) cross- 
links obtained by mass spectrometry for the SAGA complex. The mass 
spectrometry measurement was repeated twice independently with similar 
results. Totals of 396 unique intersubunit cross-links and 514 intrasubunit 
cross-links were obtained. **d**, Validated cross-links mapped onto the SAGA 
structure. Out of 396 unique intersubunit cross-links, 120 could be mapped 
onto the core-module structure, and 109 were located within the 30 Å distance 
limit for the BS3 cross-linker. Blue lines depict the cross-links with cross-linked 
sites within the 30 Å distance permitted by BS3, whereas red lines depict cross- 
links over more than 30 Å.
Extended Data Fig. 3 | Comparison of the histone-like fold in SAGA with the histone octamer, details of Taf5–Spt20 interactions, and model of the SAGA–TBP complex. Relates to data in Figs. 1–3. 

a, Comparison of the SAGA core module histone octamer-like structure with the canonical histone octamer core (PDB: 1AOI). The canonical octamer core is rendered as the colour for the SAGA octamer-like fold. 

b, Details of Taf5–Spt20 wedge interactions. Residues involved in the interactions are shown in sticks and coloured as indicated. 

c, Details of interactions between the Taf5 LisH domain and Spt20 SEP domain. Residues involved in the interactions are shown in sticks and coloured as indicated. 

d, Model of the SAGA–TBP complex. The model was generated by superposing the TBP-containing TFII Dlobe A onto the SAGA core structure. A homology model for Spt8 was generated by the I-TASSER server. 

Residues involved in the interactions are shown in sticks and coloured as indicated.
Extended Data Fig. 4 | Details of intermodule interactions. Relates to data in Figs. 1, 2. 

a, Binding interface between core and Tra1 modules. The Tra1 FAT domain (grey) is shown as a surface representation. The TIRs of Taf12 (green) and Spt20 (yellow) are shown in cartoon representation.

b, Details of the interactions depicted in a.

c, Sgf73 (turquoise) tethers the DUB module to the core module. Residues involved in the interactions are shown in sticks and coloured as indicated.

d, Sequence alignment of SAGA subunit regions involved in intermodule interactions. Conserved residues are highlighted in blue. Key residues are labelled with asterisks.* Sc, S. cerevisiae; Pp, Pichia pastoris; Sp, Schizosaccharomyces pombe.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Cryo-EM structure determination and analysis of the SAGA–nucleosome complex. Relates to data in Fig. 4. a, Exemplary cryo-EM micrograph of data collection. The micrograph is shown before (left) and after (right) denoising using Warp35. b, The 2D class averages for the SAGA–nucleosome complex. c, The 2D class averages for the DUB module–nucleosome subcomplex. d, Sorting and classification tree used to reconstruct the DUB module–nucleosome complex at 3.7 Å resolution. e, FSC between half maps of the final reconstructions of the SAGA module, Tra1 and the DUB module–nucleosome complex from SAGA–nucleosome complex data. Resolutions for the gold-standard FSC 0.143 criterion are listed. f, Angular distribution plot for all particles in the final reconstruction of the SAGA DUB module–nucleosome complex. Colour shading from blue to yellow correlates with the number of particles at a specific orientation as indicated. g, Superposition of the crystal structure of DUB-ubiquitinated nucleosome (4ZUX)12 onto the cryo-EM structure presented here. Structures are shown in cartoon and coloured as indicated. h, Comparison of the low-pass-filtered overall cryo-EM maps of SAGA and the SAGA–nucleosome complex. Densities for the HAT and DUB modules are lost on nucleosome binding to SAGA.
## Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

|                      | SAGA (EMD-10412) (PDB 6T9I) | SAGA Tral module (EMD-10413) (PDB 6T9J) | SAGA core module (EMD-10414) (PDB 6T9K) | SAGA DUB module-nucleosome (EMD-10415) (PDB 6T9L) | SAGA in nucleosome-bound state (EMD-10416) |
|----------------------|-------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------------|-------------------------------------------|
| **Data collection and processing** |                               |                                           |                                           |                                                 |                                           |
| Magnification        | 130,000                       | 130,000                                   | 130,000                                   | 105,000                                         | 105,000                                    |
| Voltage (kV)         | 300                           | 300                                       | 300                                       | 300                                             | 300                                        |
| Electron exposure (e-/Å²) | 42.45                        | 42.45                                     | 42.45                                     | 44.17                                           | 44.17                                      |
| Defocus range (µm)   | 1.25 to 2.75                  | 1.25 to 2.75                              | 1.25 to 2.75                              | 1.25 to 2.75                                    | 1.25 to 2.75                              |
| Pixel size (Å)       | 1.05                          | 1.05                                      | 1.05                                      | 1.37                                            | 1.37                                      |
| Symmetry imposed     | C1                            | C1                                        | C1                                        | C1                                              | C1                                         |
| Initial particle images (no.) | 250,368                     | 250,368                                   | 250,368                                   | 579,759                                         | 579,759                                   |
| Final particle images (no.) | 27,602                      | 27,602                                    | 27,602                                    | 113,856                                         | 86,910                                    |
| Map resolution (Å)   | 3.9                           | 3.9                                       | 3.3                                       | 3.7                                             | 6.1                                       |
| FSC threshold        | 0.143                         | 0.143                                     | 0.143                                     | 0.143                                           | 0.143                                     |
| Map resolution range (Å) | 3.2 – 8.1                     | 3.0 – 5.8                                 | 3.1 – 8.0                                 | 3.2 – 7.3                                       | –                                         |
| **Refinement**       |                               |                                           |                                           |                                                 |                                           |
| Initial models used (PDB code) | 5OJS, 6MZD, 6F3T, 2J49, 4ATG, 1SS6 | 5OJS                                      | 6MZD, 6F3T, 2J49, 4ATG, 1SS6                |                                                 |                                           |
| Model resolution (Å) | 3.3                           | 3.2                                       | 3.3                                       | 3.6                                             |                                           |
| FSC threshold        | 0.5                           | 0.5                                       | 0.5                                       | 0.5                                             |                                           |
| Model resolution range (Å) | 2.6 – 3.2                     | 2.7 – 3.2                                 | 2.8 – 3.4                                 | 3.4 – 3.8                                       |                                           |
| Map sharpening B factor (Å²) | -137.1                       | -107.119                                  | -90.9                                     | -114.6                                          |                                           |
| Model composition    |                               |                                           |                                           |                                                 |                                           |
| Non-hydrogen atoms   | 48822                         | 29551                                     | 19241                                     | 18157                                           |                                           |
| Protein residues     | 6047                          | 3617                                      | 2426                                      | 1532                                            |                                           |
| Nucleotide           | 0                             | 0                                         | 0                                         | 290                                             |                                           |
| Ligands              | 0                             | 0                                         | 0                                         | 8                                               |                                           |
| **B factor**         |                               |                                           |                                           |                                                 |                                           |
| Protein              | 58.5                          | 66.3                                      | 46.6                                      | 20.6                                            |                                           |
| Nucleic acid         | -                             | -                                         | -                                         | -                                               | 13.6                                      |
| Ligand               | -                             | -                                         | -                                         | -                                               | 523.51                                    |
| R.m.s. deviations    | 0.007                         | 0.008                                     | 0.006                                     | 0.002                                           |                                           |
| Bond lengths (Å)     | 1.295                         | 1.353                                     | 1.211                                     | 0.455                                           |                                           |
| Bond angles (°)      |                               |                                           |                                           |                                                 |                                           |
| **Validation**       |                               |                                           |                                           |                                                 |                                           |
| MolProbity score     | 1.84                          | 1.79                                      | 1.78                                      | 1.39                                            |                                           |
| Clashscore           | 6.70                          | 5.51                                      | 5.85                                      | 7.11                                            |                                           |
| Poor rotamers (%)    | 0.9                           | 0.9                                       | 0.43                                      | 0.07                                            |                                           |
| Ramachandran plot    |                               |                                           |                                           |                                                 |                                           |
| Favored (%)          | 92.48                         | 91.97                                     | 92.81                                     | 98.93                                           |                                           |
| Allowed (%)          | 7.45                          | 8.00                                      | 7.10                                      | 1.07                                            |                                           |
| Disallowed (%)       | 0.07                          | 0.03                                      | 0.08                                      | 0                                               |                                           |
### Extended Data Table 2 | Modelling of yeast SAGA subunits, domains and regions

| Subunit / Chain ID | Domain     | Residue range | Modelling method          | Notes                  |
|-------------------|------------|---------------|---------------------------|------------------------|
| Spt20 / B         | SEP        | 112-134       | *de novo* modelling       |                        |
|                   |            | 135-224       | homology-modelling: 1SS6   |                        |
|                   |            | 277-322       | *de novo* modelling       |                        |
|                   | Wedge      | 323-360       | *de novo* modelling       |                        |
|                   | TIR        | 389-416       | *de novo* modelling       |                        |
|                   | TIR        | 474-488       | *de novo* modelling       |                        |
| Spt3 / C          | HF1        | 3-86          | homology-modelling: 6M2D   |                        |
|                   |            | 140-154       | *de novo* modelling       |                        |
|                   |            | 191-209       | *de novo* modelling       |                        |
|                   | HF2        | 210-312       | homology-modelling: 6M2D   |                        |
|                   | C-term     | 313-337       | *de novo* modelling       |                        |
| Taf5 / D          | LiaH       | 56-88         | *de novo* modelling       |                        |
|                   |            | 89-125        | homology-modelling: 6M2D   |                        |
|                   | NTD        | 149-281       | Crystal structure: 2J49   |                        |
|                   |            | 430-454       | *de novo* modelling       |                        |
|                   | WD40       | 455-798       | homology-modelling: 6F3T   |                        |
|                   |            |               | & *de novo* modelling     |                        |
| Taf6 / E          | HF         | 8-77          | homology-modelling: 6F3T   |                        |
|                   |            | 78-176        | *de novo* modelling       |                        |
|                   |            | 188-220       | *de novo* modelling       |                        |
|                   | HEAT       | 221-467       | homology-modelling: 4A1G   |                        |
|                   |            |               | & *de novo* modelling     |                        |
| Taf9 / F          | HF         | 30-101        | homology-modelling: 6F3T   |                        |
|                   | C-term     | 102-149       | *de novo* modelling       |                        |
| Taf10 / G         | HF         | 67-86         | *de novo* modelling       |                        |
|                   |            | 87-196        | homology-modelling: 6M2D   |                        |
|                   | C-term     | 197-206       | *de novo* modelling       |                        |
| Ada1 / H          | HF         | 268-350       | *de novo* modelling       |                        |
|                   |            | 351-418       | homology-modelling: 6M2D   |                        |
| Taf12 / I         | TIR        | 353-413       | *de novo* modelling       |                        |
|                   | HF         | 415-486       | homology-modelling: 6M2D   |                        |
|                   | C-term     | 487-525       | *de novo* modelling       |                        |
| Spt7 / K          | HF         | 152-187       | *de novo* modelling       |                        |
|                   |            | 728-754       | *de novo* modelling       |                        |
|                   |            | 849-930       | *de novo* modelling       |                        |
|                   |            | 952-974       | *de novo* modelling       |                        |
| Sgf73 / Q         | anchor helices | 353-398     | *de novo* modelling       |                        |
|                   |            | 399-436       | *de novo* modelling       |                        |
| Tra1 / T          | Finger     | 1-950         | homology-modelling: 5OJS   |                        |
|                   | N-Clasp    | 951-1142      | homology-modelling: 5OJS   |                        |
|                   | Ring       | 1143-2445     | homology-modelling: 5OJS   |                        |
|                   | C-Clasp    | 2446-2598     | homology-modelling: 5OJS   |                        |
|                   | FAT        | 2599-3219     | homology-modelling: 5OJS   |                        |
|                   | Kinase     | 3220-3744     | homology-modelling: 5OJS   |                        |
| Chain U           | poly-alanine |            | *de novo* modelling       | Unassigned regions    |
### Extended Data Table 3 | Conservation of SAGA between yeast and human

|           | *S. c.* SAGA Subunits | *H. s.* SAGA Subunits | HHpred similarity* |
|-----------|------------------------|------------------------|-------------------|
| **HAT module** |                        |                        |                   |
| Ada2      | TADA2B                 | 0.569                  |                   |
| Ada3      | TADA3                  | 0.278                  |                   |
| Gcn5      | GCN5/PCAF              | 0.851/0.864            |                   |
| Sgf29     | SGF29                  | 0.249                  |                   |
| **DUB module** |                        |                        |                   |
| Sgf11     | ATXN7L3                | 0.356                  |                   |
| Sgf73     | ATXN7                  | 0.205                  |                   |
| Sus1      | ENY2                   | 0.518                  |                   |
| Ubp8      | USP22                  | 0.576                  |                   |
| **Core module** |                        |                        |                   |
| Taf5      | TAF5L                  | 0.578                  |                   |
| Taf6      | TAF6L                  | 0.344                  |                   |
| Taf9      | TAF9                   | 0.657                  |                   |
| Taf10     | TAF10                  | 0.688                  |                   |
| Taf12     | TAF12                  | 0.677                  |                   |
| Ada1      | TADA1                  | 0.190                  |                   |
| Spt3      | SUPT3H                 | 0.378                  |                   |
| Spt7      | SUPT7L                 | 0.167                  |                   |
| Spt8      | --                     |                        |                   |
| Spt20     | SUPT20H                | 0.295                  |                   |
| Tnl       | Tnl                    | 0.464                  |                   |

*The HHpred similarity scores are calculated between homologous regions only.*
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a
- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- [ ] A description of all covariates tested
- [ ] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [ ] A full description of the statistical parameters including central tendency (e.g. mean), or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- [ ] For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.
- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- [ ] Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- EPJ, plunk 1 (version 2.3.1), plunk 2

Data analysis

- Warp 1.0.6, RELION 3.0.5, PHENIX 1.16, COOT 0.8.9, PyMOL version 2.2.2, Chimera 1.13, XNet webserver, xVis webserver, XlinkAnalyzer version 1.1

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The electron density reconstructions of the complete SAGA complex, the Tra1 module, the core modules, and the DUB module-nucleosome complex were deposited with the EM Data Bank [accession codes EMD-10412, EMD-10413, EMD-10414, EMD-10415, and EMD-10416 respectively] and with the Protein Data Bank [accession codes 6T9J, 6T9U, 6T9K, and 6T9L, respectively].

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.


Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample size. All biochemical experiments were replicated two or more times. The crosslinking mass spectrometry measurement was replicated twice as a standard procedure for the method. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded from the analyses. |
| Replication | All attempts at replication were successful by comparing the result of each replicate. |
| Randomization | Samples were not allocated to groups. |
| Blinding | Investigators were not blinded during data acquisition and analysis because it is not a common procedure for the methods employed. |

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| n/a | n/a |
| # | Involved in the study | Involved in the study |
| □ | Antibodies | □ | ChIP-seq |
| □ | Eukaryotic cell lines | □ | Flow cytometry |
| □ | Palaeontology | □ | MRI-based neuroimaging |
| □ | Animals and other organisms | |
| □ | Human research participants | |
| □ | Clinical data | |

Eukaryotic cell lines

Policy information about [cell lines](#).

| Cell line source(s) | Saccharomyces cerevisiae strain CB10 [MATa pep4::HIS3, prb1::LEU2, ptr1::HISG, can1, ade2, trp1, ura3, his3, leu2-3,112] |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Authentication | None of the cell lines used were authenticated. |
| Mycoplasma contamination | Cell lines were not tested for mycoplasma contamination. |
| Commonly misidentified lines [See EUAC register] | No commonly misidentified cell lines were used. |