Transcription initiation involves the coordinated activities of large multimeric complexes, but little is known about their biogenesis. Here we report several principles underlying the assembly and topological organization of the highly conserved SAGA and NuA4 co-activator complexes, which share the Tra1 subunit. We show that Tra1 contributes to the overall integrity of NuA4, whereas, within SAGA, it specifically controls the incorporation of the de-ubiquitination module (DUB), as part of an ordered assembly pathway. Biochemical and functional analyses reveal the mechanism by which Tra1 specifically interacts with either SAGA or NuA4. Finally, we demonstrate that Hsp90 and its cochaperone TTT promote Tra1 de novo incorporation into both complexes, indicating that Tra1, the sole pseudokinase of the PIKK family, shares a dedicated chaperone machinery with its cognate kinases. Overall, our work brings mechanistic insights into the assembly of transcriptional complexes and reveals the contribution of dedicated chaperones to this process.
A critical step in gene expression is transcription initiation, which is controlled by many factors that typically function as part of multimeric complexes. Genetic, biochemical and structural evidence indicates that their subunits form distinct modules with specific functions. Numerous studies have characterised their activities and regulatory roles in gene expression. In contrast, much less is known about how these complexes assemble, which chaperones are required, and whether their assembly can be modulated to control or diversify their functions. Deciphering these principles is important to fully understand their structural organisation, function and allosteric regulation. Notably, chromatin-modifying and -remodelling complexes often share functional modules and therefore probably require dedicated mechanisms and chaperones for their proper assembly.

One such complex, the Spt-Ada-Gcn5 acetyltransferase (SAGA) co-activator, bridges promoter-bound activators to the general transcription machinery. In yeast, SAGA is composed of 19 subunits, which are organised into five modules with distinct regulatory roles during transcription. These include histone H3 acetylating (HAT), histone H2B de-ubiquitinating (DUB) and loading of TBP onto the promoter. A fourth module consists of a set of core components that scaffold the entire complex, most of which are shared with the general transcription factor TFIIID. Finally, the largest SAGA subunit, Tra1, is shared with another transcriptional co-activator complex, yeast NuA4, which also contains a HAT module that preferentially targets histone H4 and the H2AZ variant. Tra1 directly interacts with a diverse range of transcription factors, and recruits SAGA and NuA4 to specific promoters upon activator binding (reviewed in refs. 6,7).

Yeast Tra1 and its human ortholog, TRRAP, belong to a family of atypical kinases, the phosphoinositide 3 kinase-related kinases (PIKKs), but lack catalytic residues and are thus classified as pseudokinases. The reason for the evolutionary conservation of a typical PIKK domain architecture within Tra1 orthologs is not known. Genetic and biochemical studies indicate that Tra1 primary role is to mediate the transactivation signal from activators by recruiting SAGA and NuA4 to chromatin. It has been difficult, however, to delineate the specific contribution of Tra1 to SAGA and NuA4 architecture and activities because, to date, no clear separation-of-function alleles exist. Indeed, the mechanism by which Tra1 interacts differentially with SAGA and NuA4 remains elusive.

The fission yeast Schizosaccharomyces pombe provides a unique opportunity to address this issue because it has two paralogous proteins, Tra1 and Tra2, and each has non-redundant roles that are specific for SAGA and NuA4, respectively. Within SAGA, Tra1 has specific regulatory roles and does not contribute to its overall assembly, consistent with its peripheral position in the recent cryo-electron microscopy structure of SAGA from the budding yeast Pichia pastoris. In contrast, a recent partial structure of the yeast NuA4 complex indicates that Tra1 occupies a more central position. However, little is known about how Tra1 incorporates into the SAGA and NuA4 complexes, whether it involves similar or distinct mechanisms, and which chaperone or assembly factors are required.

Here, we show that, in S. pombe, Tra1 and Tra2 require Hsp90 and its co-chaperone, the Triple-T complex (TTT), for their de novo incorporation into SAGA and NuA4, respectively. Furthermore, proteomic, biochemical and genetic approaches identify the residues mediating Tra1 specific interaction with SAGA. Notably, we demonstrate that Tra1 contacts a surprisingly small region of the core subunit Spt20, which is both necessary and sufficient for Tra1 interaction with SAGA. Kinetic analyses of nascent Tra1 incorporation reveal that it promotes the integration of the DUB module, uncovering an ordered pathway of SAGA assembly. Finally, in contrast to the specific role of Tra1 in SAGA architecture, we show that Tra2 has a general scaffolding role in NuA4 assembly. Overall, our work brings mechanistic insights into the assembly and modular organisation of two important transcriptional co-activator complexes.

Results

The TTT subunit Tti2 contributes to Tra1 and Tra2 functions. Previous work in mammalian cells revealed that the Hsp90 chaperone TTT stabilises PIKKs, including TRRAP, the human ortholog of yeast Tra1. Three specific subunits, Tel2, Tit1 and Tit2, define the TTT complex in S. pombe, Saccharomyces cerevisiae and human cells (Supplementary Data 1). Some TTT subunits interact physically and genetically with Tra1 in S. cerevisiae and S. pombe. Fission yeasts have two paralogous genes, tra1 and tra2, and each has non-redundant roles that are specific for SAGA or NuA4, respectively. S. pombe thus offers a unique opportunity to study the specific contribution of TTT and Tra1 to SAGA and NuA4 organisation and function.

In human cells, TTT is critical for the stability of both TEL2 and TTI1 at steady state, in agreement with its stable binding to the TTT complex. We thus focused our investigations on Tti2, which we confirmed interacts with both Tra1 and Tra2 in S. pombe (Supplementary Data 1). Like Tit2, Tra2 is essential for viability, whereas tra1Δ mutants are viable. We thus developed a strategy based on inducible CreER-loxP-mediated recombination to generate conditional knockout alleles of tti2 (tti2-ΔCKO) and tra2 (tra2-ΔCKO). These strains showed β-estradiol-induced loss of Tti2 or Tra2 expression, which correlated with progressive proliferation defects, but no obvious decrease in cell viability, at least within the time frame analysed (Supplementary Figs. 1, 2). Based on these observations, tti2-ΔCKO and tra2-ΔCKO strains were induced with β-estradiol for 18 and 21 h, respectively, before further analysis.

We then performed genome-wide expression analyses of DMSO- and β-estradiol-treated tti2-ΔCKO and tra2-ΔCKO cells, compared with a CreER control strain treated identically. We also analysed tra1Δ mutants that were compared with a wild-type strain. Differential expression analysis revealed both specific and overlapping changes in each mutant (Fig. 1a–d; Supplementary Fig. 3). Specifically, gene expression changes correlated positively between tti2-ΔCKO and tra2-ΔCKO mutants, as well as between tti2-ΔCKO and tra1Δ mutants (Fig. 1a). In contrast, no correlation was observed between tra2-ΔCKO and tra1Δ mutants (Fig. 1a), as expected from their specific, non-redundant roles within either NuA4 or SAGA. A Venn diagram of the most differentially expressed genes (FC ≥ 1.5, P ≤ 0.01) reveals the extent of the overlap between all three mutants (Fig. 1b). Remarkably, over half of the Tti2-dependent genes (105/184) are also regulated by Tra1 (Supplementary Data 1). Like Tti2, Tra2 is essential for viability, whereas tra1Δ mutants are viable. We thus observed a large cluster of transcripts in which levels decrease in both tra1-ΔCKO and tra2-ΔCKO mutants (Cluster 6, 172 genes). We independently measured the expression of genes from each cluster using quantitative RT-PCR analyses and confirmed that SPCC1884.01 and SPCC9771.12 levels (Cluster 2) decrease in both tti2-ΔCKO and tra2-ΔCKO mutants (Fig. 1d), while SCC569.05c and gst2 levels (Cluster 6) decrease in both tti2-ΔCKO and tra1Δ mutants (Fig. 1c).

In S. cerevisiae, elegant biochemical and functional studies established that the primary role of Tra1 is to mediate activator-dependent recruitment of SAGA or NuA4 to specific promoters.
We thus evaluated the effect of Tti2 on the binding of the SAGA subunit Spt7 and the NuA4 subunit Epl1 to specific promoters, using chromatin immunoprecipitation (ChIP). Upon depletion of Tti2, we observed reduced occupancy of Spt7 at the pho84 and mei2 promoters and of Epl1 at the ssa2 promoter, despite normal steady-state levels (Fig. 1e, f).

In conclusion, we accumulated functional evidence that Tti2, likely as part of the TTT complex, contributes to the regulatory activities of Tra1 and Tra2 in gene expression. Therefore, similar to their active kinase counterparts, the Tra1 and Tra2 pseudokinases require the TTT cochaperone to function.

**Tti2 promotes Tra1 and Tra2 assembly into SAGA and NuA4.**
These observations prompted us to test whether Tti2, as an Hsp90 cochaperone, promotes Tra1 and Tra2 incorporation into SAGA and NuA4, respectively, as shown for human mTOR and ATR-containing complexes. For this, we affinity purified SAGA and NuA4 upon tti2 conditional deletion. Silver staining and quantitative MS analyses revealed a tenfold reduction of Tra1 from SAGA when Tti2 is depleted, as compared with control conditions (Fig. 2a).

We next tested if Tti2 promotes the de novo incorporation of Tra1 into SAGA or, rather, prevents its disassembly. For this, we took advantage of the viability of tra1Δ mutants and disrupted the tra1 promoter with a transcription terminator sequence flanked by loxP sites (RI-tra1, Supplementary Fig. 4a). With this allele, CreER-mediated recombination allows the inducible expression of Tra1 at endogenous levels. As a proof-of-principle, β-estradiol addition to RI-tra1 strains suppresses their growth defects in conditions of replicative stress, using hydroxyurea (HU) (Supplementary Fig. 4b), to which tra1Δ mutants are sensitive. Purification of SAGA from β-estradiol-treated
Fig. 1 The TTT subunit Tti2 contributes to Tra1- and Tra2-dependent gene expression. a, b RNA-seq analyses of control creER, inducible tti2 (tti2-CKO) and tra2 knockouts (tra2-CKO), tra1Δ and wild-type (WT) strains (n = 3 independent biological samples). creER, tti2-CKO and tra2-CKO cultures were supplemented with either DMSO (+tti2 or +Tra2) or β-estradiol (-tti2 or -Tra2), for either 21 h (creER and tra2-CKO) or 18 h (tti2-CKO). a Density scatter plots comparing Tti2- with Tra2-depleted cells (left, r = 0.46, P < 0.001), Tti2-depleted cells with Tra1 deletion mutants (middle, r = 0.36, P < 0.001), and Tra2-depleted cells with Tra1 deletion mutants (right, r = −0.07, P < 0.001). Statistical significance and correlation were analysed by computing the Pearson correlation coefficient. Differential gene expression analyses were performed comparing cells treated with either DMSO (control) or β-estradiol (KO), while tra1Δ mutants were compared with isogenic WT cells. Genes whose expression is regulated by β-estradiol treatment (Supplementary Fig. 3a) were filtered out. b Venn diagrams showing the overlap of differentially expressed genes (DEGs) between all mutants. Using FC ≥ 1.5, P ≤ 0.01 thresholds, 184 DEGs were filtered out.

RI-tra1 cells showed a time-dependent, progressive increase of newly synthesised Tra1 (neo-Tra1) in Spt7 puriﬁed from Tti2-HA puriﬁed cells, showing a time-dependent, progressive increase of RI-tra1 tel2-AID cells using silver staining (Fig. 3b) and quantitative MS analyses (Fig. 3d). Both approaches showed decreased interaction between newly synthesised Tra1 and afﬁnity puriﬁed Spt7 in cells partially depleted of Tel2. These results demonstrate that TTT contributes to the de novo incorporation of Tra1 into the SAGA complex.

Experiments with human cells suggested that TTT functions as an adaptor recruiting the HSP90 chaperone speciﬁcally to PIKKs16,19,31. We thus determined if Tra1 incorporation into SAGA requires Hsp90. We ﬁrst tested the effect of conditionally inactivating Hsp90 on SAGA subunit composition at steady state.
For this, we affinity purified Spt7 from hsp90–26 temperature-sensitive mutants grown at either permissive or restrictive temperature. Silver staining analysis showed that Hsp90 harbors a weaker Hsp90 mutant allele. Silver staining (Fig. 3c) and quantitative MS analyses (Fig. 3d) revealed a decrease of newly synthesised Tra1 in SAGA purified from hsp90–201 mutants, as compared with wild-type cells. Although the observed

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**Fig. 3** TTT and Hsp90 promote de novo assembly of Tra1 into SAGA. a Silver staining analysis of SAGA complexes purified upon Tra1 synthesis (neo-Tra1). Spt7-TAP RI-tra1 cells were grown to exponential phase and harvested at different time points after β-estradiol addition, as indicated (hours). SAGA was purified from a WT strain as a positive control, in which the asterisk labels steady-state Tra1 levels. Below is an anti-HA western blot of Spt7-TAP from a fraction of the input used for TAP. Ponceau red staining is used as loading control. Data are representative of four independent experiments. b Silver staining of SAGA complexes purified upon Tra1 synthesis (neo-Tra1), either in presence or absence of the TTT subunit Tel2. spt7-TAP RI-tra1 tel2-AID cells were grown to exponential phase in rich medium, supplemented with either ethanol (–IAA) or auxin (+IAA) for 16 h, and harvested 6 h after addition of either DMSO (–) or β-estradiol (+). SAGA was purified from a WT strain as a positive control, in which the asterisk labels steady-state Tra1 levels. Below are anti-HA western blotting of Spt7-TAP and Tel2-AID from a fraction of the input used for TAP. Both the TAP and AID sequences are in frame with HA epitopes. Ponceau red staining is used as loading control. Data are representative of two independent experiments. c Silver staining of SAGA complexes purified upon Tra1 synthesis (neo-Tra1), either in WT or hsp90–201 mutant strains. spt7-TAP RI-tra1 hsp90 and spt7-TAP RI-tra1 hsp90–201 cells were grown to exponential phase in rich medium and harvested 6 h after addition of either DMSO (–) or β-estradiol (+). Below is an anti-HA Western blot of Spt7-TAP from a fraction of the input used for TAP. Ponceau red staining is used as loading control. Data are representative of two independent experiments. d LC-MS/MS analyses of SAGA purifications from control spt7-TAP RI-tra1 strains, spt7-TAP RI-tra1 tel2-AID strains used in (c), and spt7-TAP RI-tra1 hsp90–201 strains used in (e). LFQ intensity ratios of newly synthesised Tra1 (neo-Tra1) were normalised to the bait, Spt7. Ratios from two independent experiments are plotted individually with the mean (black bar). Source data are provided as a Source Data file.
Mechanism of Tra1 specific interaction with SAGA. We next sought to determine how Tra1 interacts specifically with SAGA, taking advantage of the viability of tra1 mutants in S. pombe and guided by the most recent cryo-electron microscopy structure of SAGA from P. pastoris (Fig. 5a)12. Resolution of the secondary structure elements of Tra1 bound to SAGA identified a narrow
and highly flexible hinge region that was suggested to form the major, if not the single interaction surface between Tra1 and the rest of the complex. This region is located near the start of the Tra1 FAT domain and consists of about 50 residues that fold into three distinct α-helices (H1-H3, Fig. 5a). Multiple alignments of Tra1 orthologs from yeast, invertebrate and vertebrate species indicate that this region is conserved throughout eukaryotes (Fig. 5a). Interestingly, the homologous region of S. pombe Tra2, which is only present in Nua4, is more divergent, suggesting that this region might dictate SAGA binding specificity.

Deletion of a few helices within Tra1 can cause important structural rearrangements and destabilise the protein. Thus, to determine the contribution of this 50-residue region to Tra1-SAGA interaction, we swapped them with those from the closest homolog of Tra1, S. pombe Tra2, which does not interact with SAGA. We also introduced the corresponding sequence from S. cerevisiae Tra1 (Fig. 5b), which is shared between SAGA and NuA4. If we first verified that both Tra1-SpTra2 and Tra1-ScTra1 mutant proteins are expressed at levels similar to those of wild-type Tra1 (Fig. 5c). In contrast, silver staining and quantitative MS analyses revealed that the Tra1-SpTra2 hybrid is not detectable in Sp7 purifications, whereas normal levels of the Tra1-ScTra1 hybrid are observed (Fig. 5c). Similarly, a Tra1-mTOR hybrid protein is unable to copurify with SAGA (Supplementary Fig. 9a), consistent with human mTOR assembling into unrelated complexes, TORC1 and TORC2. Importantly, quantitative MS analyses show that both Tra1-ScTra2 and Tra1-ScTra1 hybrid mutant proteins efficiently copurify with Tti2 (Supplementary Fig. 9b). Thus, this region does not affect Tra1 binding to TTT and the Tra1-ScTra2 mutant protein is recognised by its cochaperone, despite being unable to interact with SAGA.

Phenotypic analyses of tra1-Sptra2 and tra1-Sctra1 strains showed that tra1-Sptra2 mutants are sensitive to HU and caffeine, similar to tra1Δ mutants, whereas tra1-Sctra1 strains show no growth defects, as compared with wild-type cells (Fig. 5d). RNA-seq analyses of tra1-Sctra1 and tra1-Sptra2 mutants revealed that the transcriptomic changes observed in tra1-Sptra2 and tra1Δ mutants correlate well ($r^2 = 0.58$), as compared with wild-type cells (Fig. 5e). In contrast, tra1-Sctra1 mutants show little changes, correlating poorly with tra1Δ mutants ($r^2 = 0.16$) (Fig. 5f).

To conclude, a 50-residue region from S. cerevisiae Tra1 complements the orthologous region from S. pombe Tra1, likely because S. cerevisiae Tra1 is present in both SAGA and NuA4. In contrast, the paralogous region from S. pombe Tra2 diverged such that it cannot interact with SAGA. Whether this region of Tra2 is responsible for its specific integration into NuA4 remains to be determined. Altogether, structural, biochemical and functional evidence demonstrates that Tra1 directly contacts SAGA through a restricted, 50-residue region located at the beginning of the FAT domain. This region of Tra1 consists of three α-helices that fold into a cup-shaped structure (Fig. 5a), which we thus coined the Cup SAGA Interacting (CSI) region of Tra1.

The SAGA subunit Spt20 anchors Tra1 into the SAGA complex. Patrick Schultz’s laboratory reported that the Tra1-SAGA hinge accommodates a putative α-helix belonging to a SAGA subunit other than Tra1. This observation encouraged us to identify the residues forming the other side of the hinge and directly contacting the Tra1 CSI region. Besides Tra1, 18 subunits form the S. pombe SAGA complex. Genetic, biochemical and structural evidence suggests that of those, Ada1, Taf12 and Spt20 are candidates to anchor Tra1 within SAGA. Silver staining analyses revealed that Tra1 is undetectable in SAGA purified from spt20Δ mutants, without any other visible changes in its overall migration profile (Fig. 6a). Spt20 is therefore essential for Tra1 incorporation into SAGA.

S. pombe Spt20 is 474-residue long and comprises an N-terminal half that contains several conserved regions, named homology boxes (HB) and a C-terminal low-complexity region (LCR) (Fig. 6b). Deletion of the Spt20 N-terminal half (residues 1–255) abolished its interaction with SAGA (Supplementary Table 1), indicating that this portion of Spt20 mediates its integration into the complex. Silver staining analyses of SAGA purified from mutants that remove various lengths of the Spt20 C-terminal LCR identified a short region of 11 residues that is crucial for Tra1-SAGA interaction (Fig. 6c). Quantitative MS analyses confirmed that Tra1 does not interact with SAGA in spt20-290 mutants, in which residues 291–474 are deleted, whereas normal levels of Tra1 are detected in spt20-300 mutants, in which residues 301–474 are deleted (Fig. 6c).

Structure prediction identified an α-helix in this region, which we coined the head interacting with Tra1 (HIT) (Fig. 6d). Silver staining and quantitative MS analyses of SAGA purified from mutants in which the Spt20 HIT region is deleted (spt20-HITΔ) confirmed its importance for Tra1 interaction (Fig. 6e). Similarly, mutating the HIT identified four residues, FIEN, that are important for Tra1 incorporation into SAGA, whereas the four positively charged RRKR residues contribute less (Fig. 6e). We verified that all Spt20 truncation, deletion and point mutants are expressed at levels comparable to those of wild-type Spt20 (Supplementary Fig. 10a) and, importantly, are present in purified SAGA complexes (asterisk in Fig. 6c, e). Furthermore, in all these mutants, Tra1 is expressed at levels similar to those observed in WT cells (Supplementary Fig. 10b). This observation suggests that unassembled Tra1 is stable, contrary to core SAGA subunits, and folded correctly, in agreement with the similarity of Tra1 structures whether alone or within SAGA.

We next evaluated the phenotype of spt20-HIT mutant strains. Similar to tra1Δ mutants, spt20-HITΔ and spt20-FIEN mutants are sensitive to HU, whereas spt20-RRKR show milder defects, as compared with wild-type cells (Fig. 6f). RNA-seq analyses of spt20Δ and spt20-HITΔ mutants revealed similar transcriptomic changes to those observed in tra1-Sptra2 and tra1Δ mutants, as compared with a wild-type strain. Comparing spt20-HITΔ with spt20Δ mutants revealed that the Spt20 HIT region contributes to the expression of only a subset of Spt20-dependent genes ($r^2 = 0.35$) (Fig. 6g), consistent with the HIT region being specifically involved in Tra1 interaction. Indeed, we observed a better correlation between spt20-HITΔ and tra1Δ mutants ($r^2 = 0.44$) (Fig. 6h). Remarkably, the strongest correlation was obtained when comparing spt20-HITΔ with tra1-Sptra2 mutants ($r^2 = 0.62$) (Fig. 6i), i.e., strains in which the hinge is mutated on either side of the same interaction surface. Altogether, these biochemical and functional approaches identified a narrow region of Spt20 that is necessary to integrate Tra1 into SAGA, likely by direct interaction with the Tra1 CSI region.

Sp20 is necessary for Tra1 incorporation in S. cerevisiae. Such a restricted and specific interaction surface might have appeared specifically in S. pombe, because Tra1 and Tra2 diverged enough to interact exclusively with either SAGA or NuA4. We thus sought to identify a homologous Sp20 HIT region in S. cerevisiae, in which a single Tra1 protein can interact with both SAGA and NuA4.

Although S. pombe and S. cerevisiae Sp20 orthologs diverged substantially, their overall domain organisation is similar. We thus focused our mutational analysis of S. cerevisiae Sp20 to a region located between the HB and the LCR (Fig. 7a). Silver
staining analyses of S. cerevisiae SAGA purified from mutants that remove various lengths of the Spt20 C-terminal LCR identified a short region of 18 residues (474–492) that is critical to incorporate Tra1 into SAGA (Fig. 7b). Quantitative MS analyses confirmed the importance of this region for Tra1 interaction with SAGA. Indeed, <3% of Tra1 is detected in Spt7 purification eluates from spt20–474 mutants, as compared with spt20–492 mutants or WT controls (Fig. 7c). Western blotting of Spt20 truncation mutants confirmed that each mutant is still present in SAGA (Fig. 7d). Finally, secondary structure prediction identified a α-helix in this region (Fig. 7a), further arguing that the Spt20 HIT region is functionally and structurally conserved between S. pombe and S. cerevisiae.
The Spt20 HIT region is sufficient for Tra1 interaction. We next asked whether the Spt20 HIT region is sufficient to interact with Tra1. For this, a peptide of about 50 residues encompassing the HIT region from either S. pombe or S. cerevisiae was immobilised on a column, through fusion to GST, and incubated with S. pombe protein extracts prepared from wild-type, tra1–Spt2a and tra1–Sctra1 strains. We observed that both recombinant Spt20 HIT fragments specifically pull-down wild-type S. pombe Tra1, as compared with GST alone (lanes 1 vs. 2, Fig. 7e). Similar levels of the Tra1–Sctra1 hybrid are recovered on the GST-HIT column (lanes 5 vs. 6, Fig. 7e). Consistent with our in vivo observations that the Tra1 C1 region mediates this interaction, lower amounts of the Tra1–Spt2a hybrid mutant are recovered on the GST-HIT column (lane 3 vs. 4, Fig. 7e). Overall, these experiments indicate that the Spt20 HIT region folds into a α-helix that is both necessary and sufficient for anchoring Tra1 in two highly divergent yeast species. We have thus elucidated the structural elements forming the narrow hinge and mediating the specific contact between Tra1 and the rest of SAGA. Notably, only a few residues are involved, in agreement with the peripheral position of Tra1 within SAGA.

Tra1 orchestrates an ordered pathway for SAGA assembly. Throughout this study, quantitative MS analyses of S. pombe SAGA purified from various mutants revealed an unexpected finding: the amount of DUB module subunits within SAGA consistently decreased when Tra1 is unassembled. For instance, we measured a reproducible decrease of both Sgf73 and Ubp8 in Spt7 purified from tra1Δ or β-estradiol-treated tti2-CKO cells, as compared with isogenic controls (Fig. 8a, b). Similarly, mutating either side of the hinge reduces the amount of both Sgf73 and Ubp8 in SAGA purifications, as observed in spt20–290, spt20–FITΔ, spt20–FIEN and tra1–Spt2a2 mutants (Hinge in Fig. 8a, b) (Supplementary Table 2). In contrast, the levels of Sgf73 and Ubp8 do not change in Spt7 purifications from spt20–300, spt20–RRKR and tra1–Sctra1 strains, in which Tra1 incorporates into SAGA (Supplementary Table 2). The other two DUB subunits, Sgf11 and Sus1, are ~10 kDa, and therefore less reliably quantified by MS. The reproducibility of this effect across distinct mutants confirmed the reproducibility of these observations (Fig. 8e). Supporting the existence of an ordered assembly pathway, comparing the relative levels of Sgf11 and Tra1 in SAGA at 4 h of induction shows that integration of the DUB module is slightly delayed compared with Tra1.

Overall, we accumulated functional and biochemical evidence supporting a model in which nascent Tra1 is recognised by the Hsp90 chaperone TTT, possibly to catalyse its folding into a mature conformation. Tra1 is assembled by direct interaction with a small region of Spt20 and then promotes the incorporation of the DUB module within SAGA (Fig. 8f).

Discussion

Many chromatin and transcription regulators function within large multimeric complexes. Deciphering the principles that govern their assembly is key to understanding their structural organisation, function and regulation. Our work brings several mechanistic insights into the de novo assembly and modular organisation of two such complexes, SAGA and NuA4. First, the Hsp90 chaperone TTT promotes Tra1 and Tra2 incorporation into SAGA and NuA4, respectively. Second, structure-guided mutational analyses elucidated the specificity of Tra1 interaction with SAGA vs. NuA4. The topology of the Tra1-SAGA interaction surface consists of a small region of the Tra1 FAT domain contacting a single α-helix of the core subunit Spt20. Third, in contrast to the general role of Tra2 in NuA4 complex formation, Tra1 specifically controls the incorporation of the DUB module into SAGA, uncovering an ordered pathway of SAGA assembly.

We show here that SAGA and NuA4 require a dedicated chaperone machinery to be fully assembled. A recent study in
human cells showed that both the TFIID-specific subunit TAF5 and its SAGA-specific paralog TAF5L require a specific chaperone, the CCT chaperonin, for their incorporation into pre-assembled modules. Our work therefore contributes to the emerging concept that dedicated chaperone machineries and ordered pathways control the de novo assembly of chromatin- and transcription-regulatory complexes.

Studies in mammals revealed that the pleiotropic HSP90 chaperone is specifically recruited to PIKKs by a dedicated cochaperone, the TTT complex, to promote their stabilisation and assembly into active complexes. In contrast, the effect of TTT on the Tra1 pseudokinase, the only inactive member of the PIKK family, is less characterised. TTT stabilises TRRAP in human cells and several studies reported physical and genetic interaction between Tra1 and TTT components in yeast. We accumulated functional and biochemical evidence that, in S. pombe, Hsp90 and TTT promote the incorporation of Tra1 and Tra2 into the SAGA and NuA4 complexes, respectively. In agreement, we found that the TTT subunit Tti2 contributes to Tra1- and Tra2-dependent gene expression, as well as...
as SAGA and NuA4 promoter recruitment. Therefore, although Tra1 is the sole catalytically inactive member of the PIKK family, it shares a dedicated chaperone machinery with active PIKK kinases for its folding, maturation, and assembly into a larger complex.

Phylogenetic analyses of PIKK orthologs in various organisms indicate that the Tra1 pseudokinase appeared early in the eukaryotic lineage, concomitantly with other PIKKs (our unpublished observations). As expected for a pseudokinase, catalytic residues diverged substantially. However, Tra1 orthologs retain the distinctive domain architecture of all PIKKs, which consists of a long stretch of helical HEAT repeats, followed by TPR repeats forming the FATC domain, preceding the FRB, PI3K-like and FATC domains. It is thus tempting to speculate that the requirement of PIKKs for a dedicated chaperone explains the selection pressure that is observed on the sequence and domain organisation of Tra1, in the absence of conserved, functional catalytic residues. For example, the short, highly conserved C-terminal FATC domain loops back close to the active site and is critical for mTOR kinase activity. Similarly, we found that the FATC domain is essential for Tra1 incorporation into SAGA (our unpublished observations), perhaps through allosteric control of the folding and positioning of the CSI region, which directly contacts SAGA.

Biochemical and functional evidence suggested that the Tra1 pseudokinase serves as a scaffold for the assembly and recruitment of the SAGA and NuA4 complexes to chromatin. S. pombe provides a unique opportunity to better understand its roles within each complex because it has two paralogous proteins, Tra1 and Tra2, and each has non-redundant functions that are specific for SAGA and NuA4, respectively. Our work indicates that, within SAGA, Tra1 has specific regulatory roles and does not scaffold the entire complex but, rather, controls the assembly of the DUB module. In contrast, Tra2 contributes to the overall integrity of NuA4. In agreement, the most recent structures of yeast SAGA and NuA4 showed different positions of Tra1 relative to other subunits. Within SAGA, Tra1 localises to the periphery of the complex and directly interacts with Spt20 (Figs. 6, 7), whereas it occupies a more central position within NuA4 and contacts several different subunits. We therefore anticipate that the single Tra1 protein found in most other eukaryotic organisms has distinct architectural roles between SAGA and NuA4 and functions as a scaffold only within the NuA4 complex.

However, what determines the distribution of Tra1 between SAGA and NuA4 remains elusive. One possibility is that SAGA- and NuA4-specific subunits compete for binding to the Tra1 CSI region. This mechanism would be similar to that described for mTOR assembly into the TORC1 and TORC2 complexes. Their structures revealed that the TORC1-specific subunit Raptor and the TORC2-specific subunit Rictor compete for binding to the same HEAT repeats of mTOR. Similarly, electron microscopy and cross-linking coupled to MS indicate that the Tra1 FAT domain makes extensive contacts with several distinct NuA4 subunits. We speculate that these interactions would sterically hinder the binding of Spt20 to the three α-helices forming the Tra1 CSI region (Fig. 5a). Higher resolution structures and further biochemical studies are required to test this hypothesis and explain why Tra1 binding to SAGA and NuA4 is mutually exclusive.

In marked contrast with S. cerevisiae and mammals, a tra1Δ deletion mutant is viable in S. pombe, enabling detailed biochemical and genetic studies that are more difficult in other organisms. Taking advantage of this opportunity, we made significant progress in our understanding of the topological organisation of the Tra1-SAGA interface. The latest structure of SAGA clearly shows that Tra1 occupies a peripheral position and interacts with the rest of the complex through a narrow and flexible surface interface, forming a hinge. Our structure–function analyses identified the residues that constitute the hinge. Specifically, a small 50-residue region of the large Tra1 protein dictates the specificity of its interaction with SAGA. The homologous region from S. pombe Tra2 diverged such that it cannot interact with SAGA. Conversely, within the hinge, a density predicted to form a helix not attributable to Tra1 was observed at the threshold used to resolve Tra1 secondary structure elements.

Here, we demonstrate that a short portion of Spt20, which we named the HIT region, is both necessary and sufficient to anchor Tra1 within SAGA. The strong dependency of Tra1 on Spt20 HIT region (Figs. 6, 7) suggests that these residues constitute the main interface between Tra1 and the rest of SAGA, allowing the construction of unique separation–of–function mutations for phenotypic and functional analyses. The exact roles of Tra1/TRRAP have been challenging to study genetically because of its presence in both SAGA and NuA4 and of its essential roles in S. cerevisiae proliferation or during mouse early embryonic development. As shown in S. cerevisiae (Fig. 7), identifying the residues mediating Tra1-SAGA interaction paves the way for addressing this issue.

Previous work suggested that Ada1 contributes to Tra1 incorporation into SAGA and that Taf12 might form part of the
Biochemical and functional evidence indicates that these subunits heterodimerize, as part of an octamer of histone folds forming the structural core of SAGA, analogous to that of TFIID. Even if specific residues of Ada1 and/or Taf12 directly contact Tra1 and stabilize its interaction with SAGA, we predict that their contribution will be minor, at least under the experimental conditions tested here. Our results demonstrate that the Spt20 HIT region is both necessary and sufficient for Tra1 incorporation into SAGA, in two highly divergent yeast species. Importantly, quantitative MS analyses show that Ada1 and Taf12 incorporation into SAGA does not require Spt20 (Supplementary Table 1). Nonetheless, it formally possible that deleting or mutating the Spt20 HIT region affects Ada1 and/or Taf12 conformation and position within SAGA, such that their putative contacts with Tra1 are weakened. Finally, analysis of SAGA conformations revealed continuous movements between Tra1 and the rest of SAGA, around the hinge.
This observation may have important implications for the allosteric regulation of SAGA activities. Indeed, such structural flexibility suggests that the interaction between Tra1 and Spt20 is dynamic. Therefore, depending on the conformation of the entire complex, Tra1 might directly interact with subunits other than Spt20, including Ada1 and Taf12. Understanding the molecular basis and the functional relevance of this flexibility is an important goal of future research projects, but it will require innovative methodological approaches. Overall, our findings open new perspectives to understand the molecular mechanism by which Tra1 modulates SAGA enzymatic activities upon binding transcription activators.

Recent seminal work established that complexes are generally assembled by ordered pathways that appear evolutionarily conserved. Biochemical analyses of SAGA in various mutants suggested that the last steps of SAGA assembly occur through an ordered pathway. Indeed, Spt20 is required for both Tra1 and DUB incorporation into SAGA, while Tra1 stabilises the DUB, but not Spt20. Conversely, the DUB module does not regulate Spt20 or Tra1 assembly. Finally, monitoring the fate of the DUB component Sgf73 upon Tra1 de novo synthesis supports a model in which Tra1 interacts with Spt20 and then promotes incorporation of the DUB module (Fig. 8f). However, Tra1 is presumably not directly recruiting the DUB module into SAGA. Recent structural analyses indicate that Tra1 does not stably contact any DUB component in most mature SAGA conformations. Rather, Tra1 might stabilise DUB incorporation during the assembly process, either through transient direct interaction or indirectly, by inducing a conformational change within Spt20 that allows SAGA-DUB interactions. Combining our work with previous structural and biochemical analyses suggests that Spt20 directly contacts the DUB anchor subunit, Sgf73, although a higher resolution structure of SAGA is needed to validate this hypothesis.

To conclude, these results contribute to our understanding of the mechanisms and pathways by which multifunctional transcription complexes assemble, which is essential to characterise their structural organisation and regulatory roles. The current model for Tra1 function postulates that it transmits the transcriptional signal from promoter-bound transcription factors to SAGA and NuA4 activities, which have critical roles in both basal and inducible RNA polymerase II transcription. Along this line, we noted that only a small number of genes require Tra1, Tra2 and Spt20 for their expression in S. pombe (Figs. 1a, b, 5e, f, 6g, and Supplementary Fig. 3). However, our RNA-seq analyses measured transcript levels at steady state. Recent work using nascent RNA-seq demonstrated that SAGA has a much more global role on RNA polymerase II transcription in S. cerevisiae. Therefore, it will be important for future studies to use such methodological approaches, in order to determine the genome-wide effects of SAGA and NuA4 on transcription rates in S. pombe. Our work opens exciting prospects for the characterisation of Tra1 exact roles during transcription initiation and of its specific contribution to SAGA and NuA4 regulatory activities.

Methods

Yeast manipulation and growth conditions. Standard culture media and genetic manipulations were used. S. cerevisiae strains were grown in YPD at 30°C to mid-log phase (~1 x 10^7 cells per ml). S. pombe strains were grown in either rich (YES) or minimal (EMM) media at 32°C to mid-log phase (~0.5 x 10^7 cells per ml). Proliferation assays were performed by inoculating single colonies in liquid media and counting the number of cells at different time points. For longer time courses, cultures were diluted to keep cells in constant exponential growth. Cell viability was assessed using 10 μL of the colorimetric dye methylene blue (319112, Sigma), which was incubated with a 50 μL suspension of exponentially growing yeast cells resuspended in PBS 1×. The number of blue-coloured dead cells was counted under a light microscope. For auxin-inducible targeted protein degradation (AID), cells were grown at 25°C and treated with either 0.5 mM indol-3-acetic acid (IAA, I2886, Sigma) or ethanol. For CreER-loxP-mediated recombination, cells were treated with either 1 μM β-estradiol (E27258, Sigma) or DMSO, for either 18 h (tti2-CKO strains) or 21 h (tra2-CKO and control creER strains), unless otherwise indicated.

Strain construction. All S. pombe and S. cerevisiae strains used are listed in Supplementary Table 4 and were constructed by standard procedures, using either chemical transformation or genetic crosses. Strains with gene deletions, truncations or C-terminally epitope-tagged proteins were constructed by PCR-based gene targeting of the respective open-reading frame (ORF) with kanMX6, natMX6 or his3MX6 cassette in a two step in vivo site-directed mutagenesis procedure. All strains used are listed in Supplementary Table 5. Transformants were screened for correct integration by PCR and, when appropriate, verified by Sanger sequencing or Western blotting. For each transformation, 2–4 individual clones were purified and analysed. Because the tti2 gene is essential for viability in S. pombe, C-terminal epitope tagging was performed in diploids, to generate heterozygous alleles. Their sporulation demonstrated that all C-terminally tagged Tti2 strains grew similarly to wild-type controls in all conditions that were tested.

Plasmid construction. Auxin-inducible degron (AID) tagging was performed using a plasmid, DHB137, which we constructed by inserting three HA epitopes in fusion with the three copies of the mini-AID sequence from pMXK15. V5-PAK tagging was performed using a plasmid, DHB123, which we constructed by inserting three V5 epitopes, S′ to the hphMX6 cassette into pFA6a-hphMX6 (Euroscarf #P30438). For GST pull-down assays, DNA fragments comprising either nucleotides +925 to +1054 from the S. pombe sp20 ORF, encoding residues Asp282 to Ala324, or nucleotides +1402 to +1611 from the S. cerevisiae SP20 ORF, encoding residues Met468 to Ala537, were synthesised and amplified. Each product was then subcloned into pGEX-4T2 (GE Healthcare Life Sciences), 3′ and in frame to the GST coding sequence, using the Gibson assembly kit (E2611L, New England Biolabs), to drive the DHB179 and DHB193 plasmids, respectively.
RT-qPCR. Reverse transcription and quantitative PCR analyses of cDNA were performed using RNA extracted from 50 mL of exponentially growing cells, as described\textsuperscript{57}, and according to the MIQE guidelines\textsuperscript{58}. Briefly, the total RNA was purified using hot, acidic phenol, and contaminating DNA was removed by DNase I digestion, using the TURBO DNA-free™ kit (AM1907, Ambion). One microgram of RNA was then reverse-transcribed (RT) at 55 °C with random hexanucleotide primers, using the SuperScript III First-Strand System (18080051, Thermo Fisher Scientific). Fluorescence-based quantitative PCR was performed with SYBR Green and used to calculate relative cDNA quantities, from the slope produced by standard curves for each primer pair, generated in each experiment. DNase-treated RNA samples were used as controls for the presence of genomic DNA contaminants. Standard curve slopes were comprised between $-3.5$ (90% efficiency) and $-3.15$ (110% efficiency), with an $r^2 > 0.9$. All primer sequences are listed in Supplementary Table 5.
**Protein extraction.** Protein extracts were prepared as described. Briefly, 20–25 mL cultures of exponentially growing cells were homogenised by glass bead-beating in a FastPrep (MP Biomedicals). Proteins were extracted using either a standard lysis buffer (WEB: 40 mM HEPES-NaOH pH 7.4, 350 mM NaCl, 0.1% NP40 and 10% glycerol) or trichloroacetic acid (TCA) precipitation. WEB was supplemented with protease inhibitors, including complete EDTA-free cocktail tablets (04693132001, Roche), 1 mM PMSE (P7626, Sigma), 1 µg per mL bestatin (B8385, Sigma) and 1 µg per mL pepstatin A (P5318, Sigma). Protein concentrations were measured by the Bradford method and used to load equal amounts of proteins across samples. Western blotting and antibodies. Western blotting was performed using the following antibodies: peroxidase-anti-peroxidase (PAP) (P1291, Sigma, 1:2000); anti-Calmodulin binding protein (CBP) (RCRP-45A-Z, ICLab, 1:500); anti-tubulin (B-5–1–2, Sigma, 1:5000); anti-FLAG (M2, F1804, Sigma, 1:1000); anti-MYC (9E10, Agro-Bio LC; 9E11, ab56, Abcam, 1:1000); anti-V5 (SVS-Pki, AbD Serotec, 1:1000); anti-HA (16B12, Ozyme, rabbit polyclonal, ab9110, Abcam, 1:1000). Protein concentrations were measured by the Bradford method and used to load equal amounts of proteins across samples. Quantification of signal intensity was performed using imaging, flow exposure or digital acquisition that were within the linear range of detection, as verified by loading serial dilutions of one sample, and analysed using ImageJ.

**Chromatin immunoprecipitation.** ChIP experiments were performed as previously described. Briefly, cell cultures were cross-linked in 1% formaldehyde for 30 min. Cells were then broken using a FastPrep (MP Biomedicals), and the chromatin fraction was released by 200–500 µL lysis buffer (CLB) buffer (50 mM HEPES-NaOH, pH 8.0, 150 mM NaCl, 0.5 mM DTT), supplemented with protease and phosphatase inhibitors. Following purifications, 10% of 2 mM EDTA eluates were concentrated and separated on 4–20% gradient SDS-polyacrylamide Tris-glycine gels (Biorad). Total protein content was determined by the Bradford method, and used to load equal amounts of proteins across samples. Western blotting was performed using the following antibodies: peroxidase-anti-peroxidase (PAP) (P1291, Sigma, 1:2000); anti-Calmodulin binding protein (CBP) (RCRP-45A-Z, ICLab, 1:500); anti-tubulin (B-5–1–2, Sigma, 1:5000); anti-FLAG (M2, F1804, Sigma, 1:1000); anti-MYC (9E10, Agro-Bio LC; 9E11, ab56, Abcam, 1:1000); anti-V5 (SVS-Pki, AbD Serotec, 1:1000); anti-HA (16B12, Ozyme, rabbit polyclonal, ab9110, Abcam, 1:1000). Protein concentrations were measured by the Bradford method and used to load equal amounts of proteins across samples. Quantification of signal intensity was performed using imaging, flow exposure or digital acquisition that were within the linear range of detection, as verified by loading serial dilutions of one sample, and analysed using ImageJ.

**Affinity purification.** Protein complexes were purified by the tandem affinity purification (TAP) method, as described previously, with minor modifications. One to four liters of exponentially growing cells were harvested, snap-frozen as individual droplets and grind in liquid nitrogen using a Freezer/Mill® (Spx SamplePrep). Protein extraction was performed in either WEB buffer or CHAPS-containing lysis buffer (CLB) buffer (50 mM HEPES-NaOH pH 7.4, 300 mM NaCl, 5 mM CHAPS, 0.5 mM DTT), supplemented with protease and phosphatase inhibitors. Following purifications, 10% of 2 mM EDTA eluates were concentrated and separated on 4–20% gradient SDS-polyacrylamide Tris-glycine gels (Biorad). Total protein content was visualised by silver staining, using the SilverQuest kit (LC6070, Thermo Fisher Scientific). For quantitative mass spectrometry analyses, 40% of 2 mM EDTA eluates were precipitated with TCA and analysed by mass spectrometry (MS). A downscaled version of the TAP procedure was used for standard co-immunoprecipitation followed by western blot analysis, as described previously.

**Mass spectrometry and data analysis.** Dry TCA precipitates from TAP eluates were denatured, reduced and alkylated. Briefly, each dry TCA precipitate sample was dissolved in 89 µL of TEAB 100 mM, and 1 µL of DTT 1 M was added before incubation for 30 min at 60°C. A vortex was added, and the sample was incubated overnight at 4°C. For the MS analysis, 2 × 10^6 ions were acquired within a maximum injection time of 60 ms and detected in the Orbitrap analyser. The 12 most intense ions with charge states ±2 were sequentially isolated to a target value of 1 × 10^4 with a maximum injection time of 45 ms and fragmented by HCD (Higher-energy collisional dissociation) in the collision cell (normalised collision energy of 28%) and detected in the Orbitrap analyser at 17,500 resolution. Raw spectra were processed with the MaxQuant environment (v1.5.0.0 or v1.5.5.1) and Andromeda for database search with label-free quantification (LFQ), match between runs and the iBAQ algorithm enabled. The MS/MS spectra were matched against the UniProt reference proteome (Proteome ID UP000000002 of S. pombe) (version 2017_10, https://www.uniprot.org/), 250 frequently observed contaminants, as well as reversed sequences of all entries. Different release versions were used, depending on the data analysis (Supplementary Table 6). Enzyme specificity was set to trypsin/P, and the search included cysteine carbamidomethylation as a fixed modification and oxidation of methionine, and acetylation (protein N-term) and/or phosphorylation of Ser, Thr, Tyr residue (STY) as variable modifications. Up to two missed cleavages were allowed for protease digestion. FDR was set at 0.01 for peptides and proteins and the minimal peptide length at 7.

**Recombinant GST and GST-HIT proteins were produced by IPTG induction of transformed BL21 Rosetta strains and purified on 100 µL of Glutathione Sepharose 4B beads (17075601, GE Healthcare Life Sciences), for 4–5 h at 4°C. After washing, beads were further incubated overnight at 4°C with 5–10 mg of S. pombe protein extracts prepared in WEB lysis buffer, before analysis by Coomassie blue staining and western blotting.**
protein (bait). For clarity purposes, this ratio was further normalised to that obtained in control strains in Fig. 5, Fig. 6, Fig. 7 and Supplementary Fig. 9.

RNA-seq and data analysis. Each growth condition or yeast strain was analysed in triplicate. RNA was extracted from 50 mL of exponentially growing cells RNA using TRIzol reagent (15596018, Thermo Fisher Scientific). DNA was removed by DNase I digestion, using the TURBO DNA-free® kit (AM1907, Ambion), and RNA was cleaned using the RNeasy Mini kit (74104, Qagen). The total RNA quality and concentration was determined using an Agilent Bioanalyzer. Transcripts were purified by polyA-tail selection. Stranded dual-indexed cDNA libraries were constructed using the Illumina TruSeq Stranded mRNA Library Prep kit. Library size distribution and concentration were determined using an Agilent Bioanalyzer. 48 libraries were sequenced in one lane of an Illumina HiSeq 4000, with 1 × 50 bp single reads, at Fasteris SA (Plan-les-Ouates, Switzerland). After demultiplexing according to their sequenced in one lane of an Illumina HiSeq 4000, with 1 × 50 bp single reads, at Fasteris SA (Plan-les-Ouates, Switzerland). After demultiplexing according to their.

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Author contributions

A.E.V., D.T. and C.F. designed, performed and analysed all experiments, except RNA-seq analyses (D.H.) and quantitative LC-MS/MS experiments (M.S.); D.H. designed the material in this article are included in the article indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Competing interests

The authors declare no competing interests.

Additional information

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