Cell Reports
Report

Mediator and SAGA Have Distinct Roles in Pol II Preinitiation Complex Assembly and Function

Xiao-Fen Chen,[1,3] Lynn Lehmann,[1,3] Justin J. Lin,[1,3] Ajay Vashisht,[1] Ryan Schmidt,[1] Roberto Ferrari,[1] Chengyang Huang,[1] Robin McKee,[1] Amber Mosley,[2] Kathrin Plath,[1] Siavash K. Kurdistani,[1] James Wohlschlegel,[1,*] and Michael Carey[1,*]
[1]Department of Biological Chemistry, David Geffen School of Medicine, University of California, BSRB 351A, 615 Charles E. Young Drive, Los Angeles, CA 90095-1737, USA
[2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Room MS1021H, 635 Barnhill Drive, Indianapolis, IN 46202, USA
[3]These authors contributed equally to this work and are co-first authors
*Correspondence: jwohl@mednet.ucla.edu (J.W.), mcarey@mednet.ucla.edu (M.C.)
http://dx.doi.org/10.1016/j.celrep.2012.10.019

SUMMARY

A key feature of RNA polymerase II (Pol II) preinitiation complexes (PICs) is their ability to coordinate transcription initiation with chromatin modification and remodeling. To understand how this coordination is achieved, we employed extensive proteomic and mechanistic analyses to study the composition and assembly of PICs in HeLa cell and mouse embryonic stem cell (ESC) nuclear extracts. Strikingly, most of the machinery that is necessary for transcription initiation on chromatin is part of the PIC. The PIC is nearly identical between ESCs and HeLa cells and contains two major coactivator complexes: Mediator and SAGA. Genome-wide analysis of Mediator reveals that it has a close correlation with Pol II, TATA-binding protein, and messenger RNA levels and thus may play a major role in PIC assembly. Moreover, Mediator coordinates assembly of the Pol II initiation factors and chromatin machinery into a PIC in vitro, whereas SAGA acts after PIC assembly to allow transcription on chromatin.

INTRODUCTION

Genes that are transcribed by RNA polymerase II (Pol II) contain chromatin modifications that facilitate initiation and early elongation (Wang et al., 2008, 2009; Agalioti et al., 2002; Li et al., 2007, 2008). These modifications often bind to ATP-dependent remodeling proteins such as the bromodomain-containing SWI/SNF and the chromodomain-containing CHD1, which mobilize nucleosomes to allow binding of the Pol II machinery (Hargreaves and Crabtree, 2011). Our groups are interested in determining how the assembly and function of the Pol II preinitiation complex (PIC) coordinate with the chromatin-modifying and -remodeling events at a promoter. To address this issue and study the mechanisms involved, we previously recreated transcription on naked DNA and chromatin templates, and captured the resulting PICs using templates immobilized on magnetic beads (Lin et al., 2011; Black et al., 2006; Johnson et al., 2002). This approach permits a detailed examination of PIC composition and function.

In our initial studies on immobilized, naked DNA templates, we found that the activator GAL4-VP16 formed a complex with the TFIID and Mediator coactivators that was necessary for efficient recruitment of Pol II and the general transcription factors (GTFs; Johnson et al., 2002), in broad agreement with the view of these coactivators as bridging factors (D'Alessio et al., 2009; Näär et al., 2001; Kornberg, 2005). On chromatin, we found that the histone acetyltransferase p300 acts in concert with the Mediator coactivator very early in PIC assembly and prior to the recruitment of the GTFs (Black et al., 2006, 2008). The p300-mediated acetylation of itself and chromatin led to p300 dissociation and allowed binding of the TFIID complex to Mediator, which facilitated assembly of the PIC.

In our early studies we employed immunoblotting to identify factors thought to be involved in PIC assembly and function. To obtain a more detailed understanding, we employed multidimensional protein identification technology (MuDPIT) to detect factors captured by the immobilized template (IT) on unmodified and H3K4-trimethylated (H3K4me3) chromatin templates (Lin et al., 2011). Our analysis revealed that a wide range of protein complexes involved in chromatin modification and remodeling were recruited to the PIC along with Mediator, TFIID, and Pol II. Importantly, SAGA, a well-studied H3 histone acetyltransferase in yeast and mammals, and a major coactivator in yeast (Baker and Grant, 2007; Nagy and Tora, 2007), was typically among the highest abundance factors. Moreover, numerous Pol II elongation complexes, such as PAF and the CDK9-containing superelongation complex (SEC), were detected (Smith et al., 2011).

After Pol II is initiated, it pauses 30–50 bp downstream of the transcription start site (TSS) through the action of DSIF and NELF. The SEC, which is recruited by Mediator (Takahashi et al., 2011), plays a postinitiation role by phosphorylating DSIF and NELF, thereby releasing the paused Pol II. Because many genes in vivo contain paused Pol II (Nechaev and Adelman, 2011), our work suggests that in these cases, Mediator's initial role may be in releasing the pause, after which it establishes a PIC to allow multiple rounds of transcription.

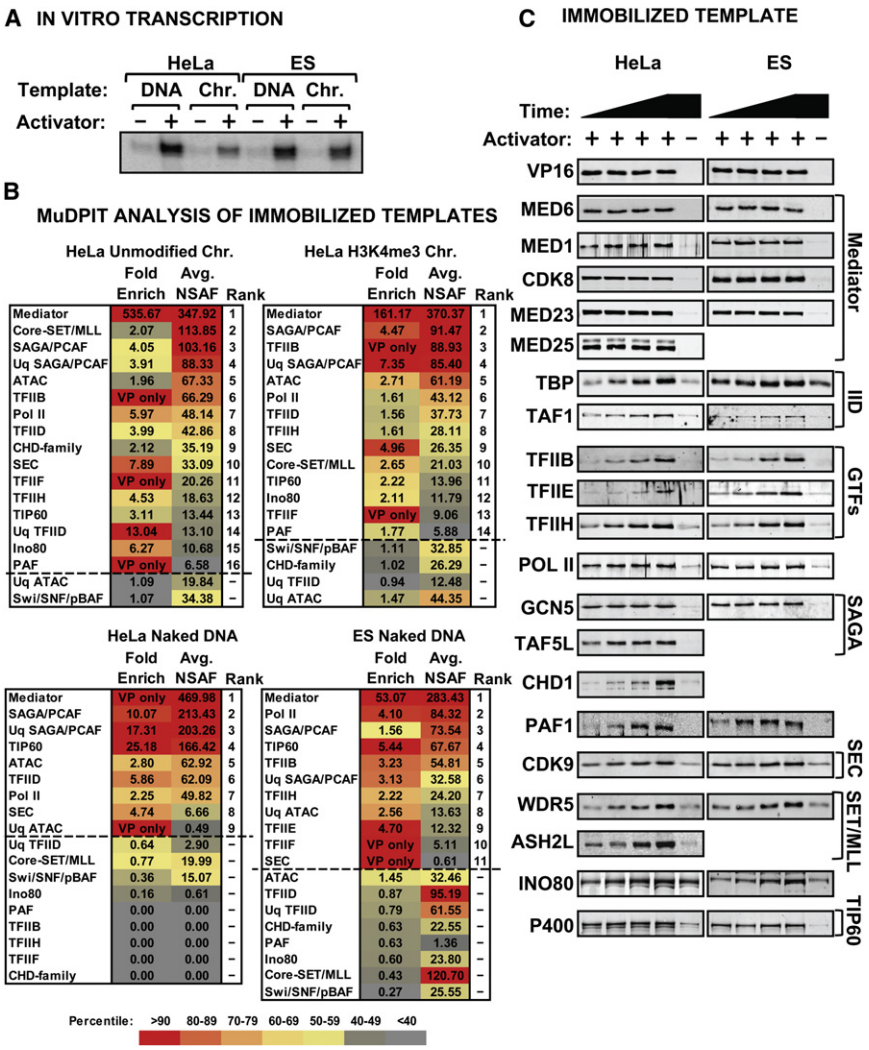

Figure 1. PIC Analysis in HeLa Cell and Mouse ESC Nuclear Extracts

(A) IVT from HeLa extract and mouse ESC nuclear extract on naked DNA and chromatin templates.

(B) MuDPIT analysis of PICs formed on naked DNA and chromatin templates in HeLa cell and ESC extracts in the presence and absence of GAL4-VP16. The primary data were analyzed by MS Sort (see Table S1) and the resulting activator-enriched ($\geq$1.5-fold; above dotted line) protein complexes were scored by average NSAF abundance. Average unique NSAFs (Uq-) for some complexes are also shown as described in the text (see Extended Experimental Procedures for a detailed description of the analysis). The fold enrichment and average NSAF values are color-coded according to the percentile of proteins shown within each experimental condition (e.g., both fold enrichment and NSAF values for Mediator are above the 90th percentile of proteins shown in the chart for each experimental condition and therefore are colored red (see key).

(C) Immunoblots representing known PIC components assembled during a 54 min time course on naked DNA templates using either HeLa cell (left) or mouse ESC (right) nuclear extract.

See also Figure S1.

The results of our initial proteomic study raised several important questions. First, are the chromatin modification and remodeling and Pol II elongation factors detected in our chromatin study components of PICs in vivo and in vitro? This bears on whether chromatin factors are general features of the PIC, whose recruitment is controlled by the activator or a major coactivator. Second, are the PICs that are formed in HeLa cells representative of PICs in other cell types, such as embryonic stem cells (ESCs), which are emerging as an exciting area of biological interest? Finally, given the abundance of SAGA, what is its role in PIC assembly and function? Previous findings in *Saccharomyces cerevisiae* showed that SAGA replaces TFIID at TATA-box-containing promoters (Bhaumik and Green, 2002; Lee et al., 2000; Huisinga and Pugh, 2004), whereas TFIID is employed at TATA-less promoters (Basehoar et al., 2004). These findings were surprising because SAGA was thought to be simply a histone acetyltransferase and deubiquitinase.

To address these questions, we employed MuDPIT to determine the composition of GAL4-VP16-stimulated PICs in vitro on DNA templates using transcriptionally active extracts from HeLa cells and mouse ESCs. We then compared the composition of the DNA PICs with that of our chromatin PICs. We found them to be highly similar, indicating that the chromatin modification and remodeling machinery is an inherent component of PICs. We then compared the in vitro PICs with Mediator-associated factors from HeLa and ESC nuclei isolated at a low salt concentration, where PIC components remain associated with chromatin (Dignam et al., 1983). The composition of the native PICs was remarkably similar to that of the in vitro PICs. Finally, we delineated the roles of SAGA and Mediator. Our data suggest that the coordinated binding of most chromatin and Pol II elongation factors, which act near the start site, is largely due to the Mediator coactivator. Importantly, we found that for the GAL4-VP16 activator, SAGA is not a coactivator in the traditional sense but functions independently of PIC assembly to allow transcription on chromatin templates.

RESULTS AND DISCUSSION

Mediator and SAGA Coactivators Are Abundant in PICs from HeLa Cells and ESCs

To study PIC assembly in HeLa versus mouse ESCs, we prepared nuclear extracts and compared their transcriptional activities using the model activator GAL4-VP16 along with a GAL4-responsive template. Figure 1A shows that ESC nuclear extracts are transcriptionally active and responsive to the activator (Lin et al., 2011). We then captured the transcriptionally

competent PICs using templates immobilized on magnetic beads (Lin and Carey, 2012). We compared unmodified chromatin, H3K4me3 chromatin, and naked DNA for HeLa nuclear extract, and naked DNA for the E14 ESC nuclear extract. The associated factors were analyzed by MuDPIT and expressed in units of normalized spectral abundance factor (NSAF; Washburn et al., 2001). Individual transcription-related proteins were sorted into complexes by an R-based program termed MS Sort, which was developed in house (see Extended Experimental Procedures). The complexes were then ranked according to activator inducibility and overall abundance. Because TFIID and SAGA share certain core subunits, we employed a "unique" average NSAF to identify whether each complex was definitively present. For cases in which unique subunit coverage of closely related complexes was too low, we relied on shared subunits or related family members to derive a score for the family of complexes (i.e., the SET1a/b and MLL1-4 complexes, and the CHD family of proteins; Nagy and Tora, 2007; Eissenberg and Shilatifard, 2010; Marfella and Imbalzano, 2007). The activator inducibility and rank abundance of the captured PICs are listed in Figure 1B. Proteins that were enriched >1.5-fold after addition of GAL4-VP16 were designated as activator stimulated. Proteins that were recruited from dialyzed nuclear extracts accurately reflect the preinitiation state because no nucleotides were added and similar recruitment data were obtained in the presence of α-amanitin and Apyrase (Figure S1).

Mediator was the most abundant activator-stimulated factor on both DNA and chromatin templates in both ES and HeLa cell extracts (Figure 1B; Table S1). Mediator is nearly identical between mice and humans (Conaway and Conaway, 2011), and 94% of the subunits were detected. SAGA was typically the second most abundant coactivator in both HeLa and ESC PICs and, like Mediator, was found on both DNA and chromatin templates (Figure 1B). SAGA contains the GCN5 HAT and a histone H2B monoubiquitination-specific protease submodule (Nagy and Tora, 2007). The abundance of SAGA on naked DNA templates was surprising. Most of our work and that of others in mammalian systems (Johnson and Carey, 2003; Johnson et al., 2002; Thomas and Chiang, 2006) suggested that Mediator and TFIID play the central roles in assembly of transcriptionally active PICs by recruiting the GTFs. Although TFIID was detected in both ESC and HeLa cell PICs, it was less abundant than SAGA and was less activator responsive in ESC extracts. Collectively, these data pointed to the possibility of a role for SAGA in PIC function.

Pol II and GTFs were also detected by MuDPIT in HeLa cell and in ESC PICs on naked DNA and chromatin. TFIIB recruits Pol II (Kettenberger et al., 2004; Chen and Hahn, 2003; Liu et al., 2010), and TFIIE and TFIIH represent the final steps in PIC assembly (Carey, 2012; Grünberg et al., 2012). Factors known to facilitate Pol II elongation, such as the CDK9-containing SEC, PAF, and TFIIF, were also found in PICs on DNA and chromatin. CDK9 promotes the release of Pol II paused by DSIF and NELF downstream of the TSS (Smith et al., 2011). PAF serves as a platform for binding of Pol II (Jaehning, 2010) but also associates with chromatin factors such as the SET1 complex (Smith et al., 2011) and CHD1 (Warner et al., 2007). Indeed, the core WDR5-RBBP5-ASH2L-DPY30 (WRAD) submodule from the SET1 trimethylase complexes was also detected (Table S1). The SWI/SNF complex was abundant on chromatin and DNA templates but not activator stimulated, whereas the Ino80 chromatin-remodeling complex was modestly activator stimulated (Figure 1B).

Due to the crude nature of HeLa cell and ESC extracts, low-abundance proteins typically fall outside the dynamic range of detection. To compensate for this, we performed immunoblotting of select unique subunits of a protein identified by MuDPIT to validate the relative amounts in a DNA versus a chromatin PIC. An analysis of DNA PICs revealed that subunits from each of the complexes were present in both HeLa cell and ESC PICs, and their activator-inducibility and binding profiles in a time course were nearly identical between the two extracts (Figure 1C). Finally, immunoblotting of PICs on chromatin showed that most factors detected by MuDPIT were present and activator inducible on chromatin as on DNA (Figure S1).

Taken together, our data suggest a model of the mammalian PIC in which an activator stimulates recruitment of GTFs and coactivators, including Mediator and TFIID, along with numerous chromatin-remodeling and -modifying enzymes. The observation that numerous chromatin and early Pol II elongation factors are detected in activator-stimulated PICs alongside Pol II and the GTFs in HeLa cells and ESCs suggests that they are important components of all mammalian PICs. This conclusion suggests that the major coactivators that control assembly of the GTFs also control chromatin events associated with initiation. To address this issue further, we examined whether Mediator and SAGA associate with PIC components on native chromatin isolated from HeLa cells and ESCs to determine the roles they play in PIC assembly and transcription in vitro.

Roles of Mediator and SAGA in PIC Assembly

Figure 2A shows a heat map of Mediator distribution in ESCs generated from published genome-wide data sets sorted by Pol II abundance (Kagey et al., 2010; Shen et al., 2012). The data reveal a remarkable similarity among the relative amounts of Mediator, Pol II, and TATA-binding protein (TBP) bound to a given gene and a close correlation with messenger RNA (mRNA) levels. This observation, along with Mediator's abundance in PICs formed in vitro, suggests that Mediator is common to many Pol II PICs in vivo, where it likely acts both in PIC assembly and in release of paused Pol II (Figure 2A). If so, then isolation of Mediator from native chromatin, as opposed to soluble nuclear extracts, should be representative of PIC composition in vivo. We isolated Med29-tagged Mediator under different stringencies from sonicated HeLa cell and ESC nuclei isolated at low salt under conditions in which the transcriptional machinery is not solubilized (Dignam et al., 1983). MuDPIT revealed that many of the proteins found in an in-vitro-assembled PIC copurified with Mediator from HeLa cell and ESC chromatin (Figure 2B). However, in contrast to the in-vitro-assembled PIC, the most abundant Mediator-associated proteins from cellular chromatin were TFIIF, TFIIB, and Pol II in HeLa cells and TFIIF, and Pol II in ESCs (Figure 2B; Table S2). The association of TFIIF and Pol II with the Mediator was previously noted (Liu et al., 2008), and TFIIB is known to associate with Pol II when it is engaged at the TSS (Kostrewa et al., 2009). TFIID is the next most abundant complex in HeLa cells. TFIIH was also detected

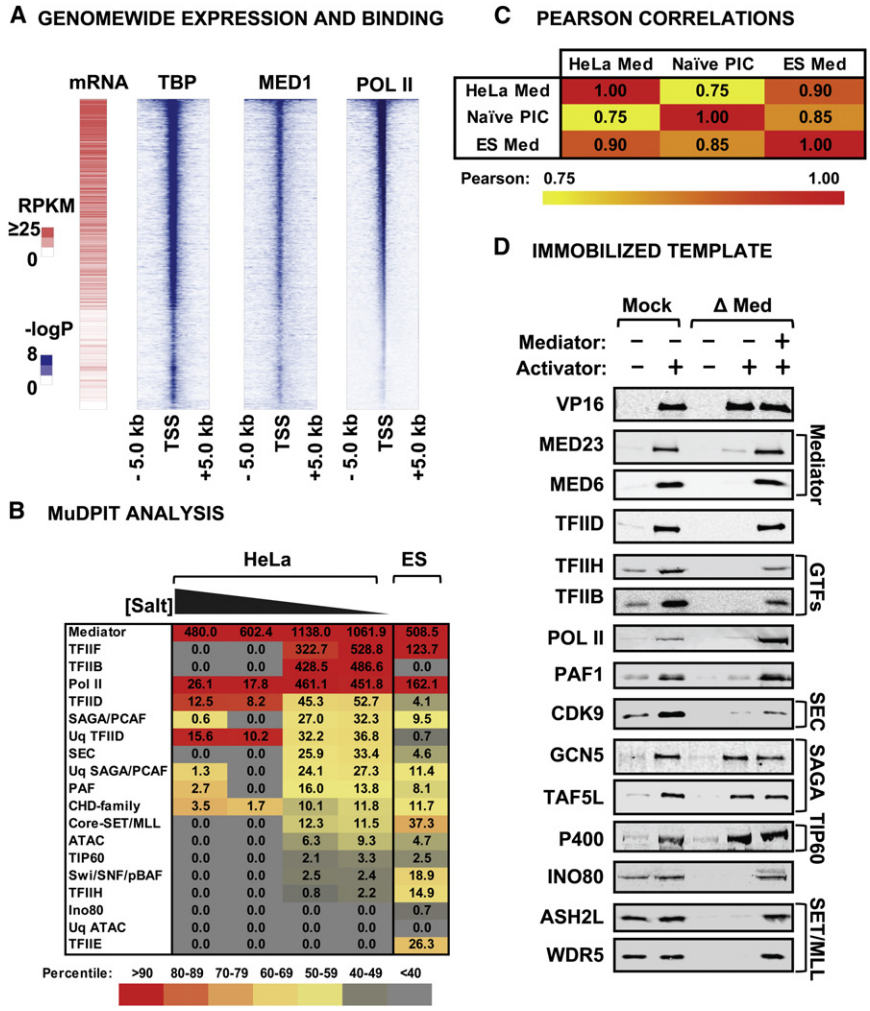

Figure 2. Mediator Composition and Function in PIC Assembly

(A) Genome-wide analysis of Mediator, TBP, and Pol II distribution in ESCs. Genes were ranked by average binding of Pol II (-log [p value]) within a 10 kb window surrounding the TSS of mouse promoters with a significant enrichment for any of the three factors ($p < 10^{-5}$). Expression data (reads per kilobase per million [RPKM]) were plotted consequently for the same ranking order.

(B) Chart depicting average NSAFs of complexes detected in MuDPIT analysis of purified FLAG-Med29 from sonicated HeLa and ESC nuclei at 100, 200, 300, and 500 mM KCl (indicated as a gradient). ESC Mediator was isolated from nuclei at 420 mM KCl. After MuDPIT and MS Sort, average NSAFs for proteins were ranked by abundance relative to the HeLa 100 mM data set. Values are color-coded according to percentile rank within all detected complexes.

(C) Pearson correlation comparison of Mediator-associated factors in unmodified chromatin and PICs assembled in vitro.

(D) Immunoblots of PIC assembly comparing the recruitment of Mediator-associated protein complexes identified by MuDPIT in Mock- or Mediator-depleted HeLa nuclear extracts. Pure Mediator was added back to depleted extracts to rescue binding. See also Figure S2.

in the HeLa cell and ESC preparations, and TFIIE was found in the ESCs, albeit at lower abundance. These observations are consistent with current models in which TFIID, Mediator, and Pol II cooperatively bind TFIIB and nucleate the assembly of other GTFs at the promoter. Other factors, such as PAF, SEC, and DSIF, were identified (reviewed in Nechaev and Adelman, 2011) along with the chromatin-modifying enzymes SAGA, SET1, and TIP60 (Figure 2B; Table S2).

A Pearson correlation comparison of the factors bound to Mediator revealed a strong similarity among HeLa cell and ESC chromatin and GAL4-VP16-stimulated PICs (Figure 2C). We conclude that the factors recruited by GAL4-VP16 in vitro are similar to those recruited by cellular activators in Mediator-associated PICs in vivo. Importantly, an IT MuDPIT analysis of ATF6α, on the *HSPA5* promoter, revealed a set of factors similar to those found in our PIC study (Sela et al., 2012).

Mediator plays a central role in recruitment of many new PIC factors. IT assays in Mediator-depleted extracts revealed that Mediator is necessary for efficient recruitment of TFIID, the GTFs, and the CDK9 subunit of PTEFb/SEC, in agreement with a previous study (Takahashi et al., 2011), and the PAF1, SET1/MLL, and Ino80 complexes. The addition of pure Mediator to the depleted extract restored binding of the affected factors, which were recruited from the depleted extract because they are not found in highly purified Mediator (Figure 2D; Figure S2). Interestingly, despite previous work suggesting a TRRAP-subunit-independent interaction between Mediator and SAGA, Mediator was not necessary for recruitment of SAGA or TIP60 (Figure 2D; Liu et al., 2008). If SAGA were a bona fide coactivator for GAL4-VP16, it should associate with GTFs or Mediator, or be important for some other aspect of PIC assembly.

Interestingly, unlike Mediator, Spt3-tagged SAGA (Martinez et al., 1998) isolated from chromatin did not contain any other factors in abundance except for the chromatin remodeler NuRD (Figure 3A; Table S3). Indeed, many of the factors detected by MS Sort were not unique; for example, the TFIID subunits detected were shared by SAGA. To our knowledge, SAGA has not been examined by chromatin immunoprecipitation (ChIP) sequencing in ESCs; however, studies in human cell lines suggest that it binds only a subset of genes, in a somewhat exclusive manner, with another GCN5-containing complex called ATAC (Krebs et al., 2011).

To further analyze SAGA's role in the PIC, we depleted it from HeLa extracts using GCN5 and TAF5L antibodies (Figure S2). Surprisingly, we found that SAGA depletion did not significantly affect GAL4-VP16-mediated PIC assembly on chromatin (Figure 3B) or DNA templates (data not shown). This result coupled

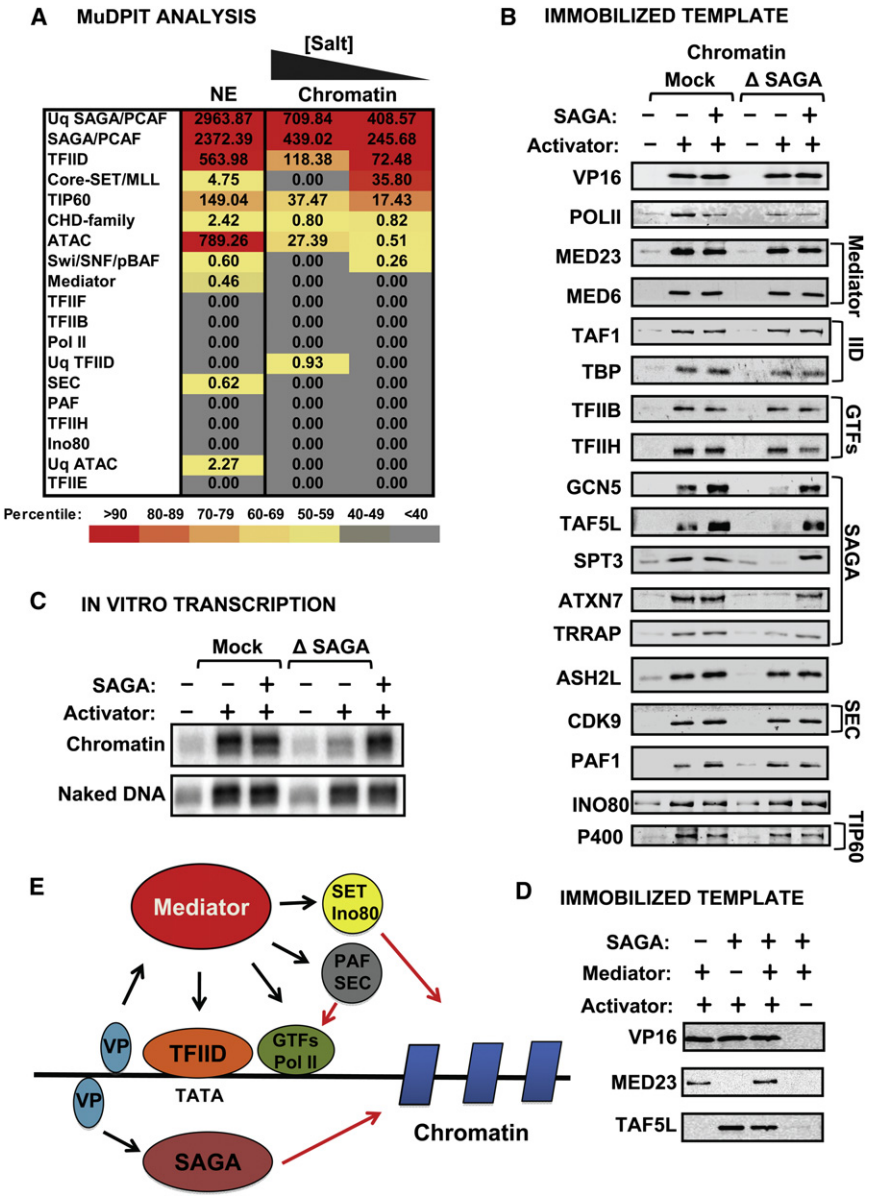

Figure 3. SAGA Composition and Role in PIC Assembly and Function

(A) Chart depicting average NSAFs of complexes detected in MuDPIT analysis of FLAG-Spt3 from sonicated HeLa nuclei at 150 and 300 mM KCl, and from soluble HeLa nuclear extract at 450 mM. Values are color-coded according to percentile rank within detected complexes.

(B) Immunoblots of PIC assembly comparing the recruitment of complexes identified by MuDPIT in Mock- or SAGA-depleted HeLa nuclear extracts plus or minus pure SAGA. Different modules of SAGA were detected using antibodies against the TRRAP, HAT/core (GCN5), SPT (SPT3), deubiquitination (ATXN7), and TAF (TAF5L) modules.

(C) IVT in SAGA-depleted extracts on naked DNA and unmodified chromatin templates plus or minus pure SAGA. A 2-fold longer exposure of transcription on chromatin is shown.

(D) Immunoblots of IT recruitment experiments with GAL4-VP16 and pure Mediator and/or SAGA.

(E) Model showing that activators recruit Mediator and SAGA independently. Mediator recruits the indicated factors. SAGA functions on chromatin. See also Figure S3.

with our proteomics data, argues that SAGA is not linked directly to PIC assembly and is therefore not a coactivator in the traditional sense, i.e., a bridging factor. Moreover, the depletion of SAGA had virtually no effect on naked DNA transcription (Figure 3C). Remarkably, chromatin transcription was strongly and reproducibly diminished by depletion and restored with pure SAGA. We conclude that SAGA does not affect PIC assembly but is required for efficient transcription of chromatin templates. It is unclear whether SAGA directly influences PIC assembly in yeast. Spt3 depletion does not affect TBP or TFIIA binding in PICs from yeast extracts (Warfield et al., 2004), but SAGA depletion abolishes activated transcription on naked DNA templates (Fishburn et al., 2005), which differs from our results in HeLa. Also, Tra1 mutants display some defects in recruitment of Pol II in vivo by ChIP assays (Knutson and Hahn, 2011), as do mutations that disrupt the Spt3-TBP interface (Mohibullah and Hahn, 2008).

Previous studies suggested a role for Mediator in SAGA recruitment to promoters (Liu et al., 2008). However, Figure 3D shows that SAGA and Mediator bind directly to GAL4-VP16 both independently and together. Moreover, Mediator recruitment is not affected by prebound SAGA, and vice versa (Figure S3). We conclude that SAGA is able to join the GAL4-VP16-assembled PIC independently of Mediator.

In summary, we determined that the primary architecture of the activator-stimulated PIC consists of high-affinity interactions between Pol II and the GTFs, driven by Mediator. Mediator also plays an essential role in recruiting several key chromatin-modifying and -remodeling complexes, such as SET1, CHD1, and Ino80, along with two major Pol II elongation complexes, PAF1 and SEC (Figure 3E). Therefore, the activator-stimulated PIC contains most of the key factors that are thought to control promoter accessibility within chromatin and early Pol II elongation. Importantly, most of the interactions are found in PICs formed on naked DNA, suggesting that promoter-bound chromatin factors are targeted to the PIC not by chromatin but by interactions with Mediator, by direct interactions with activator, or through complex networks of interactions linked to both activator and Mediator. Despite SAGA's relative abundance, its binding is directed by GAL4-VP16, and it functions after PICs are assembled (Figure 3E).

EXPERIMENTAL PROCEDURES

Recruitment and In Vitro Transcription Assays

G5E4T (Johnson and Carey, 2003) was assembled into chromatin by salt dilution and immobilized as described previously (Steger et al., 1997). IT and in vitro transcription (IVT) assays, and corresponding scaled-up reactions for MuDPIT were performed as described previously (Lin and Carey, 2012; Lin et al., 2011).

Extract and Protein Preparation

HeLa cell and mouse ESC E14 nuclear extract, and GAL4-VP16 were prepared as previously described (Dignam et al., 1983; Tantin et al., 1996). Immunodepletion of Mediator was performed as previously described (Lin et al., 2011). SAGA depletion was performed with GCN5 (Santa Cruz) and TAF5L (Sigma) antibodies. Mediator was purified as previously described (Sato et al., 2003). In parallel, Mediator was isolated from the chromatin of the low-salt nuclear fraction at the salt concentrations described in the legend of Figure 2. FLAG-Med29 was generated from the V6.5 ESC line (Beard et al., 2006) bearing a doxycycline-inducible FLAG-Med29 murine complementary DNA. SAGA was purified from nuclear extract of cells expressing FLAG-Spt3 as previously described (Martinez et al., 1998), and from the chromatin of the low-salt nuclear fraction under conditions described in the legend of Figure 3.

Genome-wide Analysis

The data sets used for genome-wide analysis were TBP-GSE22303, MED1-GSE22557, and RNA Pol II-GSM723019 from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). The expression levels for all annotated genes were determined using the GSM881355 GEO data set. All data analysis was performed as previously described (Ferrari et al., 2012)

MuDPIT Analysis of ITs and Purified Proteins

Tandem mass spectrometry spectra were collected and NSAF values were calculated as described previously (Lin et al., 2011). The average NSAFs for each complex and unique NSAFs were calculated by MS Sort by adding NSAFs for each subunit, and dividing by the total number of subunits.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, three tables, and Extended Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.10.019.

LICENSING INFORMATION

ACKNOWLEDGMENTS

We thank Joan and Ron Conaway for the Flag-Med29, and Ernest Martinez for the FLAG-Spt3 HeLa cell lines. We thank Steve Hahn for discussion. This work was supported by National Institutes of Health grants GM074701, GM089778, and GM099134 to M.C., J.W., and K.P., respectively. R.F. and S.K.K. were supported by a California Institute for Regenerative Medicine grant. M.C. wrote the paper; X.F.C., J.J.L., L.L., and C.H. performed the experiments; J.W. and A.V. performed MuDPIT; R.S. developed MS Sort; K.P. directed ESC growth and scale-up; R.F. and S.K.K. performed genome-wide analyses; and X.F.C., L.L., J.J.L., A.M., and M.C. performed data analysis.

Received: August 28, 2012
Revised: October 1, 2012
Accepted: October 24, 2012
Published: November 21, 2012

REFERENCES

Agalioti, T., Chen, G., and Thanos, D. (2002). Deciphering the transcriptional histone acetylation code for a human gene. Cell *111*, 381–392.

Baker, S.P., and Grant, P.A. (2007). The SAGA continues: expanding the cellular role of a transcriptional co-activator complex. Oncogene *26*, 5329–5340.

Basehoar, A.D., Zanton, S.J., and Pugh, B.F. (2004). Identification and distinct regulation of yeast TATA box-containing genes. Cell *116*, 699–709.

Beard, C., Hochedlinger, K., Plath, K., Wutz, A., and Jaenisch, R. (2006). Efficient method to generate single-copy transgenic mice by site-specific integration in embryonic stem cells. Genesis *44*, 23–28.

Bhaumik, S.R., and Green, M.R. (2002). Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. Mol. Cell. Biol. *22*, 7365–7371.

Black, J.C., Choi, J.E., Lombardo, S.R., and Carey, M. (2006). A mechanism for coordinating chromatin modification and preinitiation complex assembly. Mol. Cell *23*, 809–818.

Black, J.C., Mosley, A., Kitada, T., Washburn, M., and Carey, M. (2008). The SIRT2 deacetylase regulates autoacetylation of p300. Mol. Cell *32*, 449–455.

Carey, M. (2012). PICking apart Pol II initiation. Nat. Struct. Mol. Biol. *19*, 737–738.

Chen, H.T., and Hahn, S. (2003). Binding of TFIIB to RNA polymerase II: mapping the binding site for the TFIIB zinc ribbon domain within the preinitiation complex. Mol. Cell *12*, 437–447.

Conaway, R.C., and Conaway, J.W. (2011). Origins and activity of the Mediator complex. Semin. Cell Dev. Biol. *22*, 729–734.

D'Alessio, J.A., Wright, K.J., and Tjian, R. (2009). Shifting players and paradigms in cell-specific transcription. Mol. Cell *36*, 924–931.

Dignam, J.D., Lebovitz, R.M., and Roeder, R.G. (1983). Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. *11*, 1475–1489.

Eissenberg, J.C., and Shilatifard, A. (2010). Histone H3 lysine 4 (H3K4) methylation in development and differentiation. Dev. Biol. *339*, 240–249.

Ferrari, R., Su, T., Li, B., Bonora, G., Oberai, A., Chan, Y., Sasidharan, R., Berk, A.J., Pellegrini, M., and Kurdistani, S.K. (2012). Reorganization of the host epigenome by a viral oncogene. Genome Res. *22*, 1212–1221.

Fishburn, J., Mohibullah, N., and Hahn, S. (2005). Function of a eukaryotic transcription activator during the transcription cycle. Mol. Cell *18*, 369–378.

Grünberg, S., Warfield, L., and Hahn, S. (2012). Architecture of the RNA polymerase II preinitiation complex and mechanism of ATP-dependent promoter opening. Nat. Struct. Mol. Biol. *19*, 788–796.

Hargreaves, D.C., and Crabtree, G.R. (2011). ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res. *21*, 396–420.

Huisinga, K.L., and Pugh, B.F. (2004). A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. Mol. Cell *13*, 573–585.

Jaehning, J.A. (2010). The Paf1 complex: platform or player in RNA polymerase II transcription? Biochim. Biophys. Acta *1799*, 379–388.

Johnson, K.M., and Carey, M. (2003). Assembly of a mediator/TFIID/TFIIA complex bypasses the need for an activator. Curr. Biol. *13*, 772–777.

Johnson, K.M., Wang, J., Smallwood, A., Arayata, C., and Carey, M. (2002). TFIID and human mediator coactivator complexes assemble cooperatively on promoter DNA. Genes Dev. *16*, 1852–1863.

Kagey, M.H., Newman, J.J., Bilodeau, S., Zhan, Y., Orlando, D.A., van Berkum, N.L., Ebmeier, C.C., Goossens, J., Rahl, P.B., Levine, S.S., et al. (2010). Mediator and cohesin connect gene expression and chromatin architecture. Nature *467*, 430–435.

Kettenberger, H., Armache, K.J., and Cramer, P. (2004). Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIS. Mol. Cell *16*, 955–965.

Knutson, B.A., and Hahn, S. (2011). Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes. Mol. Cell. Biol. *31*, 818–831.

Kornberg, R.D. (2005). Mediator and the mechanism of transcriptional activation. Trends Biochem. Sci. *30*, 235–239.

Kostrewa, D., Zeller, M.E., Armache, K.J., Seizl, M., Leike, K., Thomm, M., and Cramer, P. (2009). RNA polymerase II-TFIIB structure and mechanism of transcription initiation. Nature *462*, 323–330.

Krebs, A.R., Karmodiya, K., Lindahl-Allen, M., Struhl, K., and Tora, L. (2011). SAGA and ATAC histone acetyl transferase complexes regulate distinct sets of genes and ATAC defines a class of p300-independent enhancers. Mol. Cell *44*, 410–423.

Lee, T.I., Causton, H.C., Holstege, F.C., Shen, W.C., Hannett, N., Jennings, E.G., Winston, F., Green, M.R., and Young, R.A. (2000). Redundant roles for the TFIID and SAGA complexes in global transcription. Nature *405*, 701–704.

Li, B., Carey, M., and Workman, J.L. (2007). The role of chromatin during transcription. Cell *128*, 707–719.

Li, Q., Zhou, H., Wurtele, H., Davies, B., Horazdovsky, B., Verreault, A., and Zhang, Z. (2008). Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. Cell *134*, 244–255.

Lin, J.J., and Carey, M. (2012). In vitro transcription and immobilized template analysis of preinitiation complexes. Curr. Protoc. Mol. Biol., *Chapter 12*, Unit 12.14.

Lin, J.J., Lehmann, L.W., Bonora, G., Sridharan, R., Vashisht, A.A., Tran, N., Plath, K., Wohlschlegel, J.A., and Carey, M. (2011). Mediator coordinates PIC assembly with recruitment of CHD1. Genes Dev. *25*, 2198–2209.

Liu, X., Vorontchikhina, M., Wang, Y.L., Faiola, F., and Martinez, E. (2008). STAGA recruits Mediator to the MYC oncoprotein to stimulate transcription and cell proliferation. Mol. Cell. Biol. *28*, 108–121.

Liu, X., Bushnell, D.A., Wang, D., Calero, G., and Kornberg, R.D. (2010). Structure of an RNA polymerase II-TFIIB complex and the transcription initiation mechanism. Science *327*, 206–209.

Marfella, C.G., and Imbalzano, A.N. (2007). The Chd family of chromatin remodelers. Mutat. Res. *618*, 30–40.

Martinez, E., Kundu, T.K., Fu, J., and Roeder, R.G. (1998). A human SPT3-TAFII31-GCN5-L acetylase complex distinct from transcription factor IID. J. Biol. Chem. *273*, 23781–23785.

Mohibullah, N., and Hahn, S. (2008). Site-specific cross-linking of TBP in vivo and in vitro reveals a direct functional interaction with the SAGA subunit Spt3. Genes Dev. *22*, 2994–3006.

Näär, A.M., Lemon, B.D., and Tjian, R. (2001). Transcriptional coactivator complexes. Annu. Rev. Biochem. *70*, 475–501.

Nagy, Z., and Tora, L. (2007). Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. Oncogene *26*, 5341–5357.

Nechaev, S., and Adelman, K. (2011). Pol II waiting in the starting gates: Regulating the transition from transcription initiation into productive elongation. Biochim. Biophys. Acta *1809*, 34–45.

Sato, S., Tomomori-Sato, C., Banks, C.A., Parmely, T.J., Sorokina, I., Brower, C.S., Conaway, R.C., and Conaway, J.W. (2003). A mammalian homolog of *Drosophila melanogaster* transcriptional coactivator intersex is a subunit of the mammalian Mediator complex. J. Biol. Chem. *278*, 49671–49674.

Sela, D., Chen, L., Martin-Brown, S., Washburn, M.P., Florens, L., Conaway, J.W., and Conaway, R.C. (2012). Endoplasmic reticulum stress-responsive transcription factor ATF6α directs recruitment of the Mediator of RNA polymerase II transcription and multiple histone acetyltransferase complexes. J. Biol. Chem. *287*, 23035–23045.

Shen, Y., Yue, F., McCleary, D.F., Ye, Z., Edsall, L., Kuan, S., Wagner, U., Dixon, J., Lee, L., Lobanenkov, V.V., and Ren, B. (2012). A map of the cis-regulatory sequences in the mouse genome. Nature *488*, 116–120.

Smith, E., Lin, C., and Shilatifard, A. (2011). The super elongation complex (SEC) and MLL in development and disease. Genes Dev. *25*, 661–672.

Steger, D.J., Owen-Hughes, T., John, S., and Workman, J.L. (1997). Analysis of transcription factor-mediated remodeling of nucleosomal arrays in a purified system. Methods *12*, 276–285.

Takahashi, H., Parmely, T.J., Sato, S., Tomomori-Sato, C., Banks, C.A., Kong, S.E., Szutorisz, H., Swanson, S.K., Martin-Brown, S., Washburn, M.P., et al. (2011). Human mediator subunit MED26 functions as a docking site for transcription elongation factors. Cell *146*, 92–104.

Tantin, D., Chi, T., Hori, R., Pyo, S., and Carey, M. (1996). Biochemical mechanism of transcriptional activation by GAL4-VP16. Methods Enzymol. *274*, 133–149.

Thomas, M.C., and Chiang, C.M. (2006). The general transcription machinery and general cofactors. Crit. Rev. Biochem. Mol. Biol. *41*, 105–178.

Wang, Z., Zang, C., Rosenfeld, J.A., Schones, D.E., Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Peng, W., Zhang, M.Q., and Zhao, K. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. Nat. Genet. *40*, 897–903.

Wang, Z., Schones, D.E., and Zhao, K. (2009). Characterization of human epigenomes. Curr. Opin. Genet. Dev. *19*, 127–134.

Warfield, L., Ranish, J.A., and Hahn, S. (2004). Positive and negative functions of the SAGA complex mediated through interaction of Spt8 with TBP and the N-terminal domain of TFIIA. Genes Dev. *18*, 1022–1034.

Warner, M.H., Roinick, K.L., and Arndt, K.M. (2007). Rtf1 is a multifunctional component of the Paf1 complex that regulates gene expression by directing cotranscriptional histone modification. Mol. Cell. Biol. *27*, 6103–6115.

Washburn, M.P., Wolters, D., and Yates, J.R., 3rd. (2001). Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat. Biotechnol. *19*, 242–247.