MOLECULAR AND CELLULAR BIOLOGY, Jan. 2006, p. 250–260 Vol. 26, No. 1
0270-7306/06/$08.00+0 doi:10.1128/MCB.26.1.250–260.2006

Drosophila Paf1 Modulates Chromatin Structure at Actively Transcribed Genes‡

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Received 29 July 2005/Returned for modification 18 September 2005/Accepted 8 October 2005

The Paf1 complex in yeast has been reported to influence a multitude of steps in gene expression through interactions with RNA polymerase II (Pol II) and chromatin-modifying complexes; however, it is unclear which of these many activities are primary functions of Paf1 and are conserved in metazoans. We have identified and characterized the *Drosophila* homologs of three subunits of the yeast Paf1 complex and found striking differences between the yeast and *Drosophila* Paf1 complexes. We demonstrate that although *Drosophila* Paf1, Rtf1, and Cdc73 colocalize broadly with actively transcribing, phosphorylated Pol II, and all are recruited to activated heat shock genes with similar kinetics; Rtf1 does not appear to be a stable part of the *Drosophila* Paf1 complex. RNA interference (RNAi)-mediated depletion of Paf1 or Rtf1 leads to defects in induction of *Hsp70* RNA, but tandem RNAi-chromatin immunoprecipitation assays show that loss of neither Paf1 nor Rtf1 alters the density or distribution of phosphorylated Pol II on the active *Hsp70* gene. However, depletion of Paf1 reduces trimethylation of histone H3 at lysine 4 in the *Hsp70* promoter region and significantly decreases the recruitment of chromatin-associated factors Spt6 and FACT, suggesting that Paf1 may manifest its effects on transcription through modulating chromatin structure.

Proper control of gene expression is necessary for the development, differentiation, and survival of the cell, and transcription regulation is a cornerstone of this process. The formation of mRNA in eukaryotes involves a complex multistep pathway wherein each step provides an opportunity for regulation. Once RNA polymerase II (Pol II) has been recruited to a promoter and initiates transcription, it must efficiently escape from the promoter-proximal region and transcribe through a gene that is covered with nucleosomes (13, 24, 43, 45). The nascent RNA must also be capped, spliced, polyadenylated, and exported to the cytoplasm before it can serve as a template for protein translation. Recent evidence from many laboratories indicates that there is a dynamic interplay between the protein complexes that carry out mRNA transcription, processing, and export, such that the efficiency of one step can have significant consequences for other steps in the pathway (3, 16, 57). For this reason, many factors that are required for the production of functional, mature RNA and were initially thought to directly stimulate Pol II transcription elongation have since been shown to elicit their primary effects on cotranscriptional processing or RNA export (e.g., reference 23). Thus, a major goal towards understanding the mechanisms of transcription regulation requires the identification of both the direct and indirect activities of the numerous factors implicated in RNA production.

The yeast Paf1 complex is one example of a factor that has been linked to a number of transcription-related activities. Yeast Paf1 is a complex of at least five polypeptides (Paf1, Rtf1, Cdc73, Leo1, and Ctr9) that has been implicated in processes as divergent as transcription initiation and elongation, modification of histone tails, phosphorylation of the Pol II C-terminal domain (CTD), RNA processing, and export (8, 9, 12, 20–22, 27, 28, 48). Although yeast Paf1 was originally thought to be an alternate mediator based upon its direct interactions with Pol II (41, 42), it has since been found to be recruited throughout the body of active genes and to associate with the elongation-competent form of Pol II (21, 26, 32, 44). Additional roles for the Paf1 complex have been suggested by the association of Paf1 with several RNA processing and export factors, such as Ccr4, the major yeast deadenylase, and Hpr1, a component of the THO complex that is involved in the export of mRNAs (8, 16).

Components of the Paf1 complex are nonessential in yeast, but mutations in Paf1 subunits confer sensitivity to 6-azauracil and generate Spt$^-$ phenotypes, which are generally thought to signify defects in transcription elongation (9, 48). In vitro transcription assays with naked DNA templates suggested that Paf1 and Cdc73 might directly stimulate transcription elongation (34); however, it is not clear what effects Paf1 has on elongation rates in vivo. In *Saccharomyces cerevisiae*, deletion of Paf1 or Cdc73 did not alter the distribution of Pol II on an active gene but dramatically decreased the chromatin immunoprecipitation (ChIP) signal observed for serine 2-phosphorylated (Ser2-P) Pol II (26). Consistent with a Ser2 phosphor-

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‡ Supplemental material for this article may be found at http://mcb.asm.org/.

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ylation defect, recruitment of 3′ cleavage and processing factors was impaired in the *paf1*Δ strain and poly(A) tail length was modestly shortened (26).

A link between the Paf1 complex and the chromatin architecture within transcribed regions has been suggested by genetic interactions between Paf1 components and Chd1, subunits of the yeast FACT complex, and histone assembly factors in the Hir/Hpc pathway (11, 44, 48). The packaging of template DNA into nucleosomes is known to represent a formidable obstacle to Pol II elongation in vitro (14, 15), an obstacle which is overcome in vivo by a number of proteins that facilitate Pol II elongation by modifying chromatin structure and/or stability. Examples of factors that have been implicated in transcription through nucleosomes are chromatin remodeling enzymes, such as Chd1 and Swi/Snf, and histone-binding proteins like Spt6 and FACT (4, 13, 44). The ensemble of these complexes appear to help disassemble nucleosomes to promote efficient Pol II transcription through bound DNA and then to reassemble nucleosomes after the passage of Pol II. Both Spt6 and FACT have recently been shown to help maintain the proper balance between assembly and disassembly of nucleosomes during active transcription by Pol II (18), with the loss of these factors leading to a net failure to reassemble nucleosomes in the wake of transcription.

The yeast Paf1 complex is required for ubiquitination of histone H2B at lysine 123 in the promoter-proximal region of activated genes (27, 49, 50). This ubiquitination event is a prerequisite for the methylation of histone H3 (at lysine residues 4 and 79) that accompanies active transcription in yeast; thus, the latter processes are defective in cells lacking functional Paf1 (27, 49, 50). In addition, the Paf1 complex has been reported to be critical for the recruitment of the yeast SET2 histone methyltransferase complex to actively transcribed genes, leading to methylation of histone H3 at residue lysine 36 (22, 54).

Although the yeast Paf1 complex has been studied extensively, a number of important questions remain unanswered. Key questions concern the nature of the interactions between the subunits of the Paf1 complex and their associations with Pol II, as well as the importance of Pol II binding in Paf1 function. A pivotal issue concerns the fact that deletion of Rtf1 or Cdc73 has been reported to reduce the association of all Paf1 components with the Pol II and chromatin yet lead to much weaker phenotypes than does deletion of the other Paf1 components (5, 26, 48). These results have led some to propose that the critical role of Paf1 occurs when the complex is not chromatin associated; however, the other potential activities of Paf1 have yet to be clearly identified (26, 33). Furthermore, the subunit composition of the Paf1 complex in human cells appears to differ from that in yeast, since the human Rtf1 protein does not appear to stably associate with the other members of the Paf1 complex (35, 55, 56).

To address these issues and to investigate the activity of Paf1-associated proteins in *Drosophila*, we have identified and characterized the *Drosophila* homologs of the yeast Paf1, Rtf1, and Cdc73 proteins. Our in vivo analyses of the *Drosophila* Paf1 complex uncover both important similarities to and differences from the reported functions of Paf1 in yeast and provide insight into the connections among histone methylation, nucleosome stability, and transcription activation in a metazoan organism. Strikingly, the *Drosophila* Paf1 homolog is a previously annotated gene that encodes an essential protein, suggesting that the role of Paf1 has evolved and become more critical in metazoans. Rtf1 is not stably associated with the *Drosophila* Paf1 and Cdc73 proteins in vivo and shows only a weak interaction with Pol II. Moreover, when Paf1-depleted cells are assayed by tandem RNA interference (RNAi)-ChIP, we observe no changes in the level of Ser2-P Pol II on the *Hsp70* gene, in contrast to results obtained with yeast. Interestingly, it appears that major effects of Paf1 depletion are the loss of H3-K4 trimethylation near the *Hsp70* promoter and a significant decrease in the recruitment of Spt6 and FACT to the body of the *Hsp70* gene, suggesting that *Drosophila* Paf1 may coordinate the activities of elongating Pol II with factors that maintain the proper chromatin architecture during transcription.

MATERIALS AND METHODS

Plasmid construction, recombinant protein purification, and antibody production. The *Drosophila* genes encoding Paf1 (CG2503), Rtf1 (CG10955), and Cdc73 (CG11990) were identified by searching the *Drosophila* genome database for the *Drosophila* homologs of the *S. cerevisiae* genes. Recombinant proteins were expressed and purified and antibodies generated against them as described in the legend to Fig. S1 in the supplemental material.

Indirect immunofluorescence. Unless otherwise noted, polytene chromosomes were prepared from third-instar larvae of *Drosophila* strain 87E (53), which contains an *Hsp70* transgene at locus 87E. Where indicated, larvae were heat shocked at 36.5°C before the chromosomes were spread and stained with antibodies. The H14 antibody (1:50 dilution; Covance) against Ser5-P Pol II and H5 antibody (1:50 dilution; Covance) against Ser2-P Pol II were used as previously described (25). Lab stocks of Paf1 and Rtf1 antibodies were added at the following dilutions: rabbit anti-Paf1, 1:20; rat anti-Paf1, 1:5; rat anti-Rtf1, 1:50. Analysis was performed by using a Zeiss Axioplan 2 microscope and OpenLab 3.0.7 or Volocity imaging software.

RNAi and ChIP. RNAi and ChIP were performed as described in reference 1. Briefly, *Drosophila* S2 cells were treated with double-stranded RNA (dsRNA) targeting β-galactosidase (LacZ, as a negative control), Paf1, Rtf1, Cdc73, or both Paf1 and Rtf1. After 72 h of treatment, cells were heat shocked at 36.5°C before harvesting. For RNA analysis, cells were heat shocked for 30 min before total RNA was isolated and *Hsp70* levels determined by quantitative real-time reverse transcription-PCR. Stable ribosomal protein (RpL32) gene *rp49* was used as an internal standard as previously described (1, 29). Values shown represent three to five independently RNAi-treated samples that were each analyzed in duplicate with an oligo(dT) reverse primer; however, we obtained nearly identical results with gene-specific reverse primers. Error bars represent the standard error of the mean (SEM).

Each value for ChIP experiments was derived from IPs of at least three independent cell cultures that were either cross-linked directly after harvesting (non-heat shocked) or after the given time of heat induction. The results of ChIP experiments are plotted against the *Hsp70* gene position for each primer pair (with the position denoting the center of each pair) as follows. Primer set 1, position −154, extends from −200F to −108R. Primer set 2, position +58, extends from +4F to +112R. Primer set 3, position +379, extends from +334F to +423R. Primer set 4, position +946, extends from +872F to +1019R. Primer set 5, position 1952, extends from +1925F to +1978R. Primer set 6, position +2669, extends from +2631F to +2706R. Primer set 7, background 32 kb, is downstream of the 3′ end of *Hsp70* at 87C. Primers for other genes are selective for either the promoter or downstream region. *Hsp26* primer pairs are located at −22F to +63R and +580F to +667R. Actin5c is at −3F to +90R and +1781F to 1848R. β-Tubulin is at −70F to +11R and 2075F to 2152R. Glyceraldehyde-3-phosphate dehydrogenase is at −57F to +13R and +958F to +1030R. dMyc (diminutive) is at +190F to +274R and +1861F to +1919R. Primer sequences are available upon request.

ChIPs employed affinity-purified rabbit Rtf1 antibody, rabbit Paf1 serum, and affinity-purified rabbit Cdc73 antibody. Other antibodies were lab stocks that have been described previously. SSRP1 antibody, a gift from Susumu Hirose, (was used at 4 μl per IP); H5 (Covance) was used at 2 μl per IP; anti-H4 (ChIP

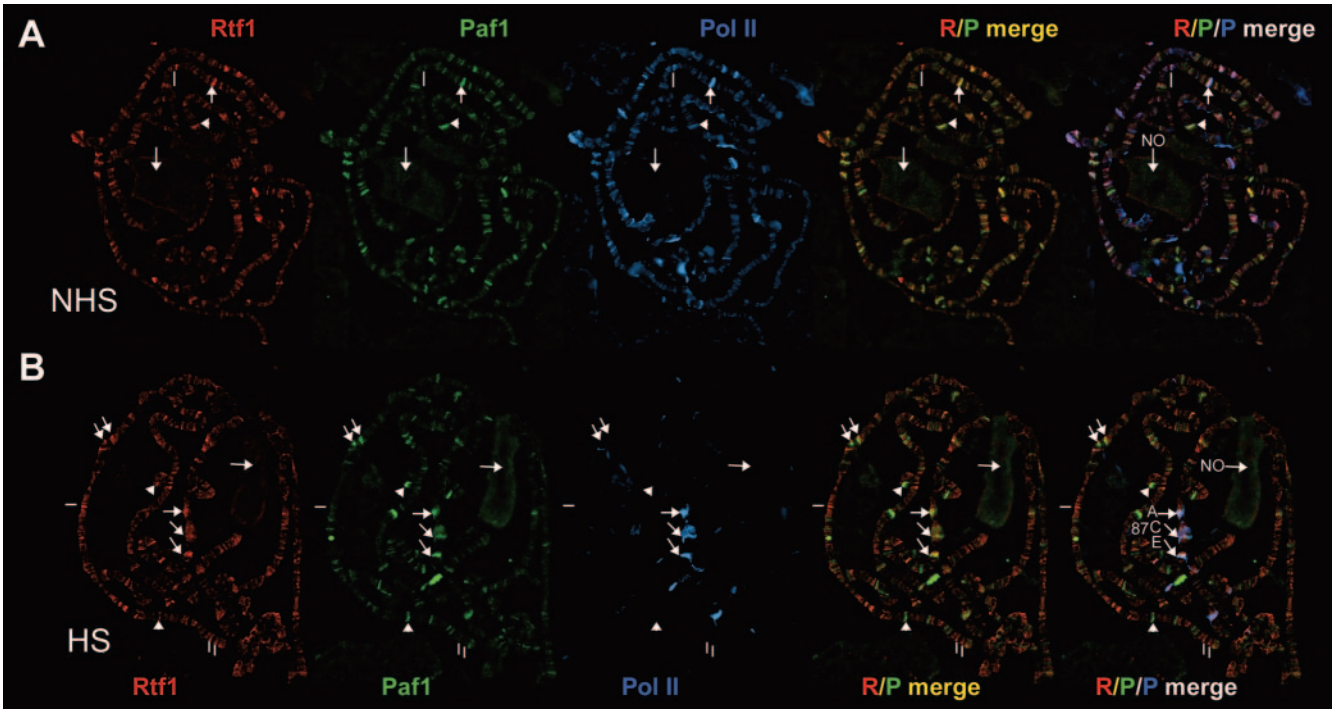

FIG. 1. Paf1 and Rtf1 colocalize with each other and with active Pol II on *Drosophila* polytene chromosomes. (A) Chromosomes were costained with antibodies to Rtf1 (red), Paf1 (green), and Ser5-P Pol II (H14 antibody, blue). The merge shows the complete overlap of Paf1 and Rtf1 distributions on chromosomes. The reddish bands (marked with thin white lines) in the merge of Rtf1 and Paf1 (R/P merge) show more Rtf1 than Paf1 staining; however, close inspection of all such bands shows also the presence of Paf1. Likewise, the greenish bands (marked with an arrowhead) in the merge of Rtf1 and Paf1 (R/P merge) show more Paf1 than Rtf1 staining; however, close inspection of all such bands shows also the presence of Rtf1. The R/P/P merge shows the extensive overlap of Rtf1 and Paf1 and Ser5-P Pol II distributions on chromosomes (for example, the white arrow points to a developmental puff labeled strongly by all three antibodies.). A full complement of mapped sites labeled at puff stage 9 is provided in Table S1 in the supplemental material. Also noted is the labeling of the nucleolus organizer (NO). (B) Chromosomes from heat-shocked larvae were costained with antibodies to Rtf1 (red), Paf1 (green), and Ser5-P Pol II (H14 antibody, blue). Major *Hsp70* heat shock loci at 87A and 87C and the transgene at 87E are show by the labeled arrows. The arrow, arrowhead, and line labels are as in panel A. NHS, non-heat shocked; HS, heat shocked.

grade; Abcam) was used at 10 μl per IP; and anti-trimethylated-H3-K4 (Upstate) was used at 15 μl per IP.

IPs. Whole-cell extracts were prepared from 10^9 *Drosophila* S2 cells that were lysed in a buffer containing 0.5% NP-40, 50 mM HEPES (pH 7.6), 250 mM potassium glutamate, 5 mM EDTA, 1 mM dithiothreitol, and Roche Complete protease inhibitors. After lysis, a sample of the soluble material was removed for input and the remainder was divided into four aliquots that were mixed with protein A-agarose beads, either alone or with 12 μg affinity-purified rabbit antibodies against Paf1, Rtf1, or Cdc73. After thorough washing, immunoprecipitated material was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and proteins transferred to nitrocellulose membranes. Membranes were probed with rat antibodies against Paf1 and Cdc73, a rabbit antibody against Rtf1, and a guinea pig antibody targeting the largest subunit, Rpb1 Pol II (6).

RESULTS

Paf1 localizes with transcriptionally engaged Pol II on polytene chromosomes. The *Drosophila* homologs of the yeast Paf1, Rtf1, and Cdc73 proteins were identified by performing BLAST searches of the *Drosophila* genome with the sequences of the *S. cerevisiae* proteins. Surprisingly, the *Drosophila* homolog of Paf1 is a previously annotated gene named antimeros (*atms*, CG2503). Mutations in this gene have been described and are recessive lethal, indicating that although Paf1 is not essential in yeast, it performs a critical function in a higher eukaryote. Antibodies generated against the *Drosophila* homologs of the yeast Paf1, Rtf1, and Cdc73 proteins (see Materials and Methods) each recognized primarily a single band of the predicted size (see Fig. S1 in the supplemental material).

The global localization of the *Drosophila* Paf1 and Rtf1 proteins was investigated by analysis of polytene chromosomes. Immunostaining of chromosomes with an antibody raised against Rtf1 (Fig. 1A, red) or Paf1 (green) each showed many discrete bands of strong, moderate, and weak intensities. Merging of these patterns (R/P merge of red and green) reveals colocalization of the two proteins over the entire chromosome, signifying that *Drosophila* Paf1 and Rtf1 are localized to the same regions when they are chromatin associated. However, we noted that the intensity of staining of Paf1 and Rtf1 was not constant over the chromosomes (in Fig. 1A, the arrowhead designates an example of a site at which the Paf1 signal is more intense than the Rtf1 signal and the line designates a site where the Rtf1 signal is stronger than the Paf1 signal), which suggests that these two proteins may not uniformly exist in a complex with a constant 1:1 stoichiometry (see below).

To compare the pattern of Paf1 staining with that of active transcription, chromosomes were costained with an antibody that recognizes phosphorylated, transcriptionally engaged Pol II (Fig. 1A, Ser5-P Pol II, blue). The overlay of this signal with

that from Paf1 and Rtf1 staining shows nearly complete colocalization of all three proteins at sites along the chromosomes (R/P/P merge, last panel). To systematically investigate the localization of the Paf1 protein on chromosomes, we performed fine mapping of a number of polytene spreads that were costained with antibodies against both Paf1 and Ser5-P Pol II (see Table S1 in the supplemental material). Importantly, Paf1 is found at all transcriptionally active sites and all the Paf1 banding corresponds to active loci. This effectively complete colocalization of Paf1 with phosphorylated Pol II suggests that Paf1 is globally involved in gene expression and indicates that all of the chromatin-associated Paf1 activities are performed at actively transcribed loci.

Paf1 and Rtf1 are rapidly recruited to active heat shock loci. Although global colocalization of Paf1 with phosphorylated Pol II was observed during larval development, evidence for direct involvement of Paf1 in transcription requires demonstration of dynamic recruitment of the Paf1 complex to a transcription locus upon activation. Also, the time course of recruitment relative to other transcription factors could provide clues to the stage in gene expression where Paf1 acts. Therefore, we analyzed whether Paf1 and Rtf1 are recruited to heat shock loci upon heat induction. *Drosophila* salivary glands were heat shocked for 20 min, and the spread chromosomes were costained with antibodies against Rtf1 (Fig. 1B, red), Paf1 (green), and Ser5-P Pol II (blue). As shown in Fig. 1B, Paf1 and Rtf1 colocalize broadly over the chromosome (R/P merge); however, there are many sites where the relative levels of Rtf1 and Paf1 differ (Rtf1 > Paf1, depicted by lines; Paf1 > Rtf1, shown by arrowheads). Nonetheless, both Rtf1 and Paf1, like Pol II, were robustly recruited to the heat shock loci (R/P/P merge, heat shock genes at 87A and -C and an *Hsp70*-LacZ transgenic locus at 87E; for DNA staining, see Fig. S2 in the supplemental material). These results strongly support the conclusion that Paf1 and Rtf1 participate in active transcription or a tightly coupled process. However, while the staining for Pol II effectively disappeared from developmentally active loci upon heat shock, a significant proportion of the signal for Rtf1 and Paf1 remained at other sites on the chromosomes. These results suggest that although Paf1 and Rtf1 are recruited to heat shock loci upon gene activation, they do not simply dissociate from genes with Pol II, as is seen for a number of other elongation factors (2, 37), which raises the possibility that these proteins might associate with transcriptionally active loci through interactions with factors other than Pol II.

To further investigate Paf1 recruitment to heat shock loci, we performed a time course study of heat shock treatment, investigating specific recruitment to a transgenic *Hsp70*-LacZ locus (see Fig. S2B in the supplemental material). One characteristic of the *Hsp70* promoter is the presence of a transcriptionally engaged Pol II that has stalled, or paused, within the initially transcribed region. Although this stalled Pol II has been shown to be phosphorylated at Ser5 of the CTD prior to heat shock, phosphorylation of Ser2 and release of this Pol II into the body of the gene depend upon heat induction (24). Although no Ser2-P Pol II or Paf1 was detected at the *Hsp70* transgene prior to heat induction, after only 5 min of heat shock, there were high levels of Pol II Ser2-P and Paf1 observed at the *Hsp70* gene. Moreover, both Paf1 and Pol II were detected throughout the decondensed *Hsp70* puff after 15 min of heat shock, suggesting that Paf1 travels into the body of the gene along with elongating Pol II.

High-resolution mapping by ChIP shows Paf1 association with the *Hsp70* transcription unit in a pattern indistinguishable from that of Spt6 and FACT. We performed ChIP assays with Paf1, Rtf1, and Cdc73 antibodies to better define their localization at *Hsp70*. As mentioned above, the uninduced *Hsp70* gene is characterized by an open chromatin architecture upstream of the promoter and the presence of engaged Ser5-P Pol II that is stalled within the promoter-proximal region (6, 24). In agreement with previous results, we detected Pol II along with elongation factors Spt5 and TFIIS at the *Hsp70* promoter before heat shock (Fig. 2B; references 1 and 2). However, Paf1, Rtf1, and Cdc73 could not be detected within this or any other *Hsp70* gene region prior to heat induction (Fig. 2B). After a 10-min heat shock, Paf1, Rtf1, and Cdc73 were robustly recruited to the *Hsp70* gene (Fig. 2C). Interestingly, all three proteins were recruited much more strongly to the middle of the *Hsp70* gene than to the promoter-proximal region.

To further map the spatial distribution of Paf1 at *Hsp70*, we analyzed the ChIP material at intervals throughout the *Hsp70* gene, extending from the upstream region to sequences just past the principal site for cleavage and polyadenylation (the schematic in Fig. 2A shows the locations of primer pairs). These results reveal that Paf1, Rtf1, and Cdc73 are predominantly recruited downstream from the promoter region (Fig. 2D). These patterns of distribution are distinct from that of Pol II, since Pol II levels are nearly maximal in the promoter-proximal region (Fig. 2E, Pol II distribution, squares; Paf1 distribution, circles). Instead, the localization of Paf1 after a 10-min heat shock is almost identical to that observed for the chromatin-associated transcription factors Spt6 and FACT (Fig. 2F and data not shown). Both Spt6 and FACT have been shown previously to be recruited to the activated *Hsp70* gene, beginning near the position of the first nucleosome within the transcribed region, at approximately +200 (37).

To determine whether the pattern of Paf1 recruitment would resemble that of Pol II at an earlier time point in transcription activation, we repeated these experiments with material that was cross-linked after a short, 2.5-min heat shock. As shown in Fig. 2G, the Pol II complexes that are released from the promoter-proximal region upon heat shock are just beginning to reach the 3′ end of *Hsp70*, while significant quantities of additional Pol II have been recruited to the promoter region. However, even under these conditions, Paf1 signals are extremely low at the promoter and the Paf1 recruitment is maximal in the middle of the gene (Fig. 2G). To determine whether we could detect Paf1 associated with the promoters of other genes, we analyzed the levels of Paf1 at a number of constitutively active genes (e.g., actin, tubulin). While we detected significant levels of Pol II, we were unable to detect Paf1 associated at any of the promoter regions tested (see Fig. S3A in the supplemental material).

Importantly, the distribution of Paf1 after 2.5 min of heat shock is nearly identical to that of FACT subunit SSRP1 and Spt6 (see Fig. 2H; see Fig. S3B in the supplemental material). The similar patterns of recruitment of Paf1 and these nucleosome-modifying factors suggest that these proteins might in-

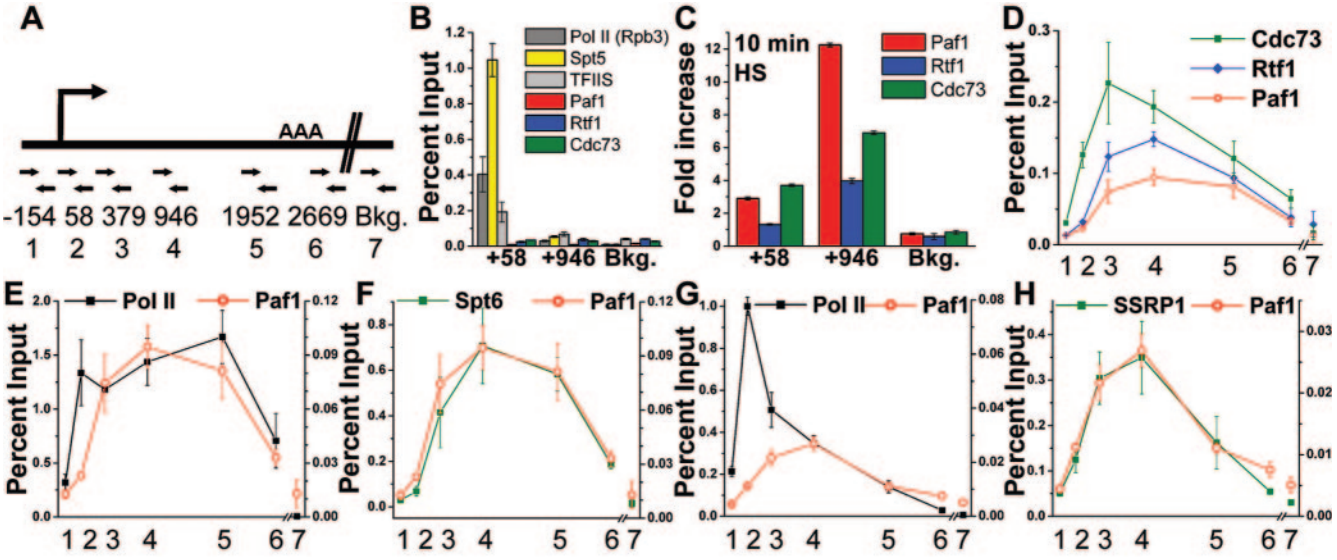

FIG. 2. Paf1 is not present at the uninduced *Hsp70* gene but is rapidly recruited throughout the body of the gene upon heat shock. (A) Locations of primer pairs used for high-resolution mapping of factor distribution at *Hsp70*. The schematic represents the *Hsp70* gene, with the arrow depicting the promoter (+1) and AAA showing the approximate location of the cleavage and polyadenylation site (+2358). The center nucleotide position of each primer pair is given. The background (Bkg.) (primer pair 7) represents an intergenic region 32 kb downstream of *Hsp70* at 87C. Graphs in panels B to H represent the percentage of the input material retrieved in each IP or the *n*-fold increase in the ChIP signal upon heat shock plotted against the positions of the individual primer pairs. Error bars represent the SEM ($n = 3$ to 5 independent ChIP samples). (B) ChIP signal plotted as the percentage of the input for Pol II (Rpb3), Spt5, TFIIS, Paf1, Rtf1, and Cdc73 within the *Hsp70* promoter region (+58), the middle of the *Hsp70* gene (+946), and the background primer set. (C) The *n*-fold increase in the ChIP signal observed upon 10 min of heat shock relative to that in uninduced cells for the promoter (+58) and coding (+946) regions of *Hsp70*. Background values are shown for comparison. (D) Patterns of Paf1, Rtf1, and Cdc73 distribution at the induced *Hsp70* gene after a 10-min heat shock. The percentage of the input obtained by amplification of immunoprecipitated material with each primer pair is shown versus the primer location on the *x* axis, as depicted in panel A. (E and F) Paf1 distribution on the *Hsp70* gene after a 10-min heat shock (red circles; percentage of the input is on the right *y* axis) plotted with the distributions of Pol II (E, black squares; the percentage of the input is on the left *y* axis) and Spt6 (F, green squares; the percentage of the input is on the left *y* axis). (G and H) Observed distribution of Paf1 (red circles, right *y* axis), Pol II (G, black squares, left *y* axis), or SSRP1 (H, green squares, left *y* axis) after a 2.5-min heat shock for each position along the *Hsp70* gene.

teract with each other in the context of actively elongating Pol II, as has been previously reported for yeast (21, 48).

Paf1 and Rtf1 are required for maximal induction of *Hsp70*. Given that Paf1 is associated with active *Hsp70* and has been implicated in modulating transcription efficiency at some genes, we tested the functional relevance of Paf1 to the induction of the *Hsp70* gene. By RNAi, we specifically depleted Cdc73, Paf1, Rtf1, or both the Paf1 and Rtf1 proteins together in *Drosophila* S2 cells. After 72 h of RNAi treatment, samples were heat shocked for 30 min before harvesting of cells for isolation of total RNA. Western blot analysis of cell lysates confirmed that the targeted proteins were efficiently and specifically depleted by this procedure (Fig. 3A, top; Cdc73, data not shown). By using quantitative real-time PCR to measure *Hsp70* levels in each sample, we found that the Paf1 and Rtf1 proteins are essential for optimal production of *Hsp70* RNA (Fig. 3B) but failed to see an effect of Cdc73 depletion on *Hsp70* levels (data not shown). Although these results may indicate that Cdc73 does not play a critical role in *Hsp70* gene activation, we recognize the caveat that the level of Cdc73 depletion may not have been sufficient to reveal a role for Cdc73. Nonetheless, the effect of Paf1 depletion is more dramatic than depletion of Rtf1, in agreement with the severity of their respective mutant phenotypes in yeast (42, 48). Moreover, the transcription defect caused by depleting Paf1 is not enhanced by codepletion of Rtf1, which suggests that these proteins function in a similar pathway when associated with elongating Pol II.

***Drosophila* Rtf1 does not stably associate with Paf1 and Cdc73.** The unequal staining of Paf1 and Rtf1 at a number of chromosomal loci observed by polytene immunofluorescence raised the possibility that the two *Drosophila* proteins might not always be present in a tight complex. Moreover, recent work characterizing the human Paf1 complex suggested that human Rtf1 did not form a stable complex with other Paf1 complex components (35, 55, 56). Therefore, to investigate the proteins that associate with Paf1, Rtf1, and Cdc73 in *Drosophila* cells, we immunoprecipitated each of the three proteins from *Drosophila* S2 cell extracts. Immunoprecipitated material (and that from a no-antibody control) was probed for the presence of Paf1, Rtf1, Cdc73, and Pol II. Figure 4A shows that the rabbit antibody against Paf1 or Cdc73 efficiently immunoprecipitated both proteins. In contrast, neither the Paf1 nor the Cdc73 antibody efficiently precipitated Rtf1. Conversely, the Rtf1 antibody was able to immunoprecipitate its target, but neither Paf1 nor Cdc73 was detectable at high levels in the anti-Rtf1-immunoprecipitated material. To determine which of these proteins might anchor the *Drosophila* Paf1 complex to the polymerase during transcription, we then probed these samples for the presence of Pol II with an antibody that recognizes the core (not the CTD) of Rpb1 (6). While none of the immunoprecipitated proteins appeared to coprecipitate

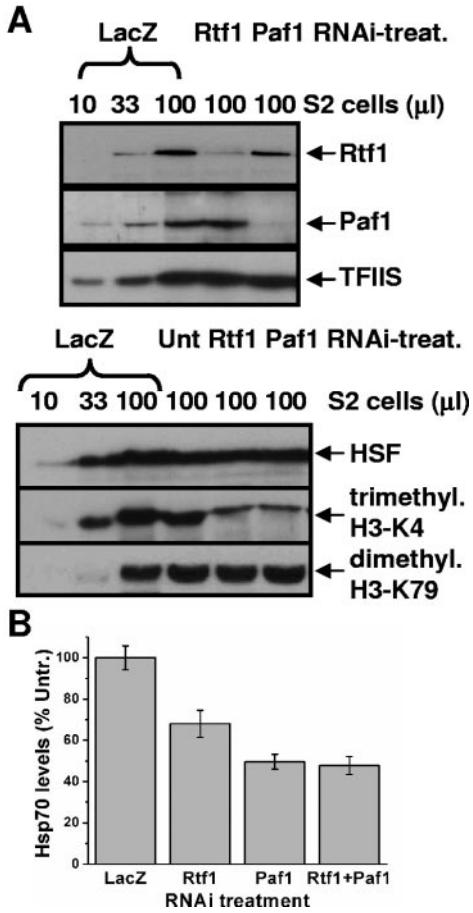

FIG. 3. Depletion of Paf1 or Rtf1 by RNAi decreases levels of induced *Hsp70* RNA. *Drosophila* S2 cells were treated (treat.) with dsRNA targeting Cdc73, Paf1, Rtf1, or both Paf1 and Rtf1 or were not treated (Unt). dsRNA corresponding to β-galactosidase (LacZ) was used as a control for the nonspecific effects of RNAi treatment. (A) Western blots of the given volumes of RNAi-treated cells were probed with various antibodies. Top: Paf1 (1:2,000), Rtf1 (1:2,000), or transcription factor TFIIS (1:3,000; as a loading control). Bottom: heat shock factor (HSF) (1:1,000; as a loading control), trimethylated (trimethyl.) H3-K4 (1:1,000; Upstate), or dimethylated (dimethyl.) H3-K79 (1:5,000; Upstate). (B) RNAi-treated cells were heat shocked, total RNA was isolated, and *Hsp70* levels were analyzed by reverse transcription and quantitative PCR. Values shown represent induced *Hsp70* levels (corrected by the levels of *rp49* [as an internal control]), expressed as a percentage of the quantity of *Hsp70* RNA obtained from untreated cells that were heat shocked and analyzed simultaneously. Untr., untreated.

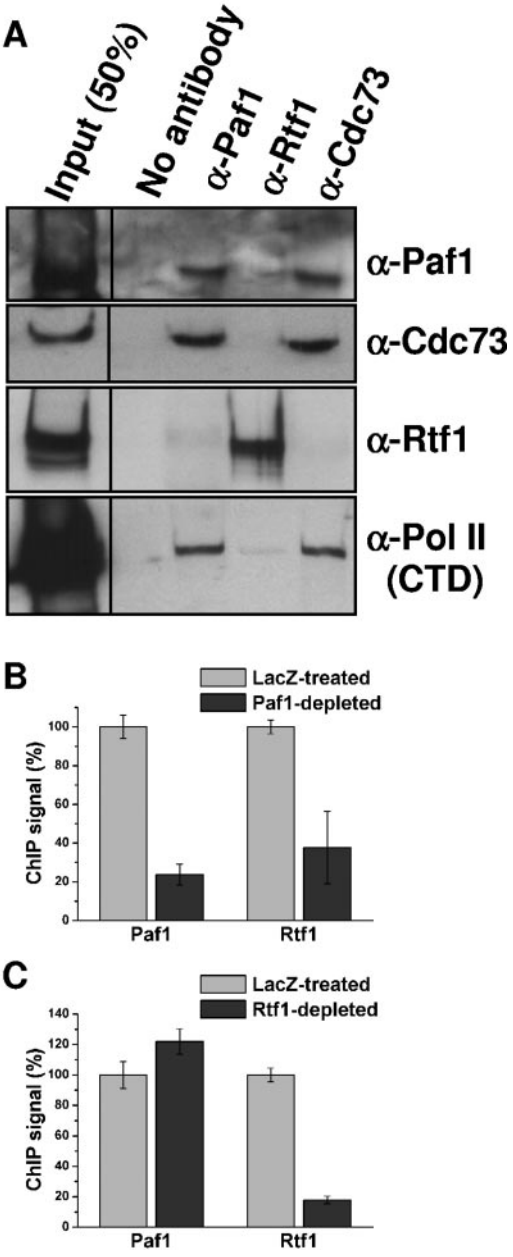

FIG. 4. *Drosophila* Rtf1 does not stably associate with Paf1 and Cdc73 and does not affect the recruitment of Paf1 to an active gene. (A) *Drosophila* cell extracts were incubated with affinity-purified rabbit antibodies against Paf1, Rtf1, and Cdc73 or with no antibody as a control. An aliquot of the input and the immunoprecipitated material were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes, which were probed for the presence of specific proteins with rat or guinea pig antibodies against Paf1, Cdc73, and Pol II or the rabbit antibody against Rtf1 as denoted at the right. (B and C) Antibodies against Paf1 and Rtf1 were used to immunoprecipitate ChIP material from LacZ-treated and Paf1-depleted (B) or Rtf1-depleted (C) cell cultures. Values (ChIP signal) shown are normalized such that the average value from LacZ-treated samples for each antibody is set at 100%. Results given are for the middle of the *Hsp70* gene, at position +946 (primer pair 4). Error bars on all graphs represent the SEM, where $n = 3$ independent RNAi-treated cell cultures.

the bulk of Pol II, there were considerable levels of Pol II in both the Paf1 and Cdc73 immunoprecipitated samples and a small but detectable level in the material associated with Rtf1. Thus, although the Paf1 complex in *Drosophila* seems to lack a stably bound Rtf1 subunit, Paf1, Cdc73, and Rtf1 might associate in the context of actively transcribing Pol II.

Depletion of Rtf1 does not reduce the association of Paf1 with the active *Hsp70* gene. Since the *Drosophila* Paf1 complex appears to be distinct from yeast Paf1, we sought to further elucidate the nature of the interactions between *Drosophila* Paf1 or Rtf1 and elongating Pol II by tandem RNAi-ChIP. We depleted either Paf1 or Rtf1 by RNAi and then performed

ChIP on these samples after a 10-min heat shock (when Paf1 levels are at their maximum). Quantification of the ChIP signal in the middle of the *Hsp70* gene (primer pair 4, centered at +946) reveals that depletion of Paf1 leads to a decrease in the Paf1 signal on the *Hsp70* gene. Surprisingly, we also observed a dramatic decrease in Rtf1 levels at *Hsp70* when Paf1 was depleted (Fig. 4B), in contrast to the situation reported in yeast. Moreover, we noted that the Paf1 levels detected on the *Hsp70* gene in Rtf1-depleted cells were comparable to those in the LacZ-treated sample, indicating that *Drosophila* Paf1 can associate with elongating Pol II in the absence of Rtf1 (Fig. 4C). These results are strikingly different from those obtained by Jaehning and colleagues with yeast, where Paf1 ceases to interact with Pol II in the absence of Rtf1 (26, 33). However, in yeast, Rtf1 is tightly associated with the Paf1 complex, whereas this does not appear to be the case in humans or *Drosophila* (35, 55, 56). Moreover, these results are consistent with both the magnitude of the effects of Paf1 and Rtf1 depletion on *Drosophila Hsp70* transcription and the severity of the phenotypes displayed by the *paf1*Δ and *rtf1*Δ yeast strains, where *paf1*Δ has much more pronounced defects.

Depletion of Paf1 or Rtf1 does not affect Ser2-P Pol II levels on *Hsp70* but does affect the recruitment of FACT and Spt6. To survey for the additional effects of Paf1 and Rtf1 depletion on the recruitment and distribution of Pol II and transcription factors at *Hsp70*, we performed a series of ChIP assays with RNAi-treated *Drosophila* cells. Analysis of Pol II distribution at *Hsp70* in cross-linked material from LacZ or Paf1 RNAi-treated cells (10-min heat shock) showed no significant differences in either the distribution or the density of Pol II (Rpb3) as a result of Paf1 depletion (Fig. 5A; primer locations are shown in Fig. 2A). However, we note that under these conditions, the density of actively transcribing Pol II throughout the *Hsp70* gene is extremely high (approximately one Pol II every 100 bp), so that it might be difficult to detect a modest decrease in the rate of transcription elongation (which would further increase Pol II density if the initiation rate remained unchanged). Although we did detect an approximately fivefold reduction in the ChIP signal for Paf1 in the Paf1-depleted cells (Fig. 4B), we did not observe a change in the levels of transcription factor Spt5 or the Ser2-P form of Pol II in the Paf1-depleted samples (Fig. 5B, levels shown are for primer pair 4, or position +946 within the *Hsp70* gene). Thus, the reduced accumulation of *Hsp70* RNA observed in Paf1-depleted cells does not appear to result from (i) a decrease in the overall recruitment of Pol II or transcription elongation factor Spt5 to the gene or (ii) inefficient phosphorylation of Ser2 on the Pol II CTD.

To confirm these results, similar ChIP experiments were performed with independent LacZ-treated or Paf1-depleted samples that were heat shocked for only 5 min before cross-linking. The data shown in Fig. S4 in the supplemental material are in agreement with the above data, demonstrating that depletion of Paf1 does not considerably alter the recruitment or progression of Ser2-P Pol II along the *Hsp70* gene, nor does it affect the association of transcription elongation factor Spt5.

Because the distribution of Paf1 so closely mimicked that of Spt6 and the SSRP1 subunit of FACT during heat shock activation, we next investigated whether depletion of Paf1 altered the recruitment of these factors to *Hsp70*. Interestingly, significantly lower levels of both Spt6 and SSRP1 are observed at the *Hsp70* gene in the absence of Paf1 (Fig. 5B), suggesting that the association of Paf1 with elongating Pol II may facilitate the recruitment of Spt6 and FACT to a transcribed gene. To ensure that the decrease in the Spt6 and SSRP1 signals obtained at *Hsp70* in ChIP assays was not due to a global depletion of these proteins, we performed Western blot assays (see Fig. S5A in the supplemental material) which revealed that Spt6 and SSRP1 were present at normal levels in Paf1-depleted cells.

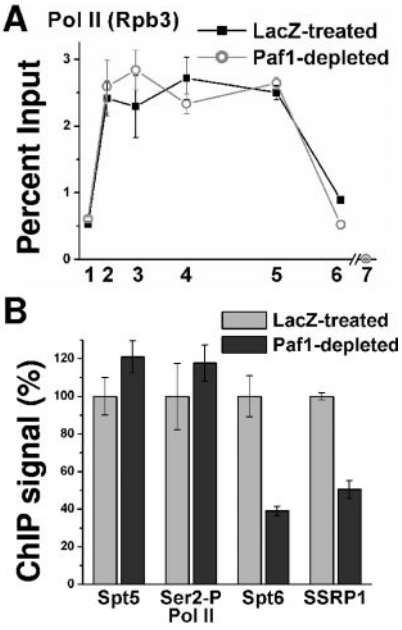

FIG. 5. Paf1 is required for maximal recruitment of nucleosome-associated factors Spt6 and FACT. Cross-linked (ChIP) material from LacZ or Paf1 RNAi-treated cells that were heat shocked for 10 min was analyzed as described in the legends of Figs. 2 and 4. (A) The levels of Pol II (Rpb3), expressed as percentages of the input, are shown for LacZ-treated (squares) versus Paf1-depleted (circles) cells at intervals throughout the *Hsp70* gene (positions shown on *x* axis, as described in Fig. 2A). (B) Antibodies against transcription elongation factor Spt5, the Ser2-P form of Pol II, or the chromatin-associated factors Spt6 and SSRP1 were used to immunoprecipitate ChIP material from LacZ-treated (light gray bars) or Paf1-depleted (dark gray bars) cells. Values (ChIP signal) shown are normalized such that the average value from LacZ-treated samples for each antibody is set at 100%. Results given are for the middle of the *Hsp70* gene, at position +946 (primer pair 4). Error bars on all graphs represent the SEM, where $n = 3$ to 5 completely independent RNAi-treated cell cultures.

To test for a role for Rtf1 in recruiting chromatin-associated factors to active genes, ChIP assays were also performed with Rtf1-depleted cells. These results reveal a modest decrease in the recruitment of Spt6 and SSRP1 to the active *Hsp70* gene in the absence of Rtf1, while levels of Pol II and Spt5 remain largely unchanged (see Fig. S5B in the supplemental material). The more modest decreases in Spt6 and SSRP1 levels resulting from Rtf1 depletion are in agreement with the more subtle defects in *Hsp70* transcription upon depletion of Rtf1 compared to depletion of Paf1.

Histone H3 trimethylation at lysine 4 is dependent on Paf1 and Rtf1. To further address the function of *Drosophila* Paf1 in transcription, we investigated several previously determined

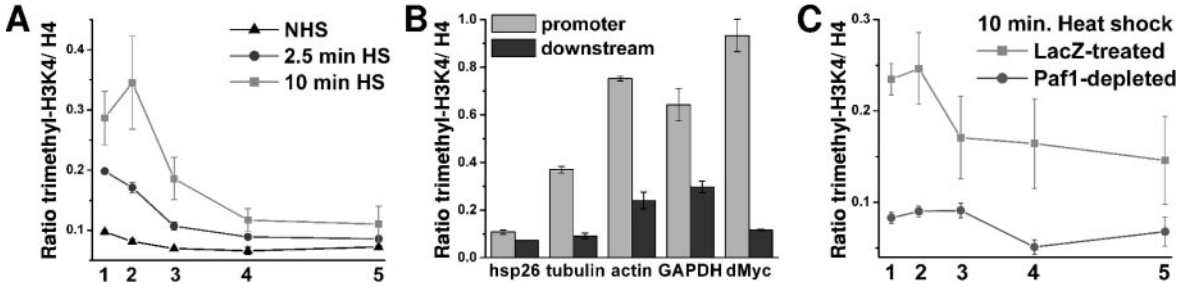

FIG. 6. Depletion of Paf1 abolishes methylation of histone H3 at lysine 4 during gene activation. (A) ChIP assay evaluating the level of H3-K4 trimethylation present at uninduced *Hsp70* (triangles) or after 2.5 min (circles) or 10 min (squares) of heat shock (HS). Values shown are a ratio of the trimethyl-H3-K4 levels divided by the signal from IP of histone H4. Values are averages of three independent samples, with error bars representing the SEM. The numbers on the *x* axis represent the *Hsp70* primer pairs used, as shown in Fig. 2A. (B) Primers were designed to amplify the promoter (light gray) or downstream (dark gray) region of a second heat-induced gene, *Hsp26*, and several active *Drosophila* genes. Values are averages of the ratio of the trimethylated H3-K4 signal divided by the levels of cross-linked histone H4 from two or three independent non-heat-shocked (NHS) samples. Error bars represent the range of values obtained. (C) The ratio of trimethylated H3-K4 over the H4 signal in LacZ-treated (squares) or Paf1-depleted (circles) samples that were heat shocked for 10 min is shown for each primer pair (*Hsp70* positions are shown on the *x* axis, as described in Fig. 2A). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

phenotypes from yeast. The yeast Paf1 complex has been implicated in the ubiquitination of histone H2B (at residue K123) within promoter-proximal nucleosomes and in the methylation of histone H3 at residues K4, K36, and K79 (22, 27, 49, 50). Unfortunately, antibodies against ubiquitinated *Drosophila* H2B are unavailable; thus, we were unable to directly probe the role of *Drosophila* Paf1 in this process. However, we did investigate levels of methylation of histone H3 in S2 cells depleted of Paf1 and Rtf1 by RNAi. As shown in Fig. 3A (bottom), depletion of the Paf1 or Rtf1 protein dramatically decreases the amount of trimethylated H3-K4 detected in bulk histones by Western blot analysis; in contrast, we did not detect any change in the di- or trimethylation of H3 at K79 (Fig. 3A, bottom; see Fig. S5A in the supplemental material) or in the dimethylation of H3 at residue K36 (data not shown). In *S. cerevisiae*, deletion of Paf1 components did not alter bulk levels of dimethylated H3-K36 but did diminish levels of H3-K4 trimethylation and H3-K79 dimethylation (27). Thus, although our data for H3-K4 and H3-K36 methylation are in agreement with results from yeast, the lack of an effect of Paf1 depletion on H3-K79 di- or trimethylation in S2 cells indicates that this histone modification is not directly dependent on Paf1 activity in *Drosophila*.

H3-K4 trimethylation at the *Hsp70* gene is rapid and promoter region specific. Having demonstrated a role for *Drosophila* Paf1 in maintaining normal levels of trimethylated H3-K4, we wanted to probe the connections among Paf1, H3-K4 trimethylation, and transcription activation, in particular since this methylation event is linked with transcribed genes in other metazoans (36, 38, 39). As a first step, we made use of the rapid activation of heat shock genes to investigate the kinetics of H3-K4 trimethylation. By using ChIP, we compared the levels of trimethyl-H3-K4 before heat shock with the signals obtained after a 2.5- or 10-min heat shock (Fig. 6A). The results were normalized against the signal obtained with an antibody recognizing the globular domain within histone H4 in order to account for both the paucity of histones within the *Hsp70* promoter region (10, 51, 52) and the net loss of histone cross-linking observed upon gene activation (47). As shown in Fig. 6A, the relative levels of trimethylated H3-K4 are very low prior to heat shock and are constant across the gene. However, the trimethyl-H3-K4 levels increase very rapidly upon heat induction and continue to increase as a function of time after gene activation (compare 2.5-min heat shock [blue line] with 10-min heat shock [red line]), in agreement with a recent observation (46). We also note that the trimethyl-H3-K4 signal is primarily detected near the *Hsp70* promoter and upstream region. To test the generality of the promoter-proximal localization of H3-K4 methylation in *Drosophila*, we analyzed the distribution of the trimethyl-H3-K4 signal at a number of additional genes. Performing quantitative PCR with primer pairs that amplified either the promoter region or downstream sequences of each gene revealed that they uniformly possess significantly higher levels of trimethyl-H3-K4 near their promoters than in the coding regions (Fig. 6B). In contrast, *Hsp26* possesses very little trimethyl-H3-K4 signal at any gene region prior to heat shock.

Paf1 is required for H3-K4 trimethylation at *Hsp70* during gene activation. To test whether the loss of Paf1 alters H3-K4 trimethylation during gene activation, we investigated this methylation event in cells treated with dsRNA targeting LacZ (as a control) or Paf1. RNAi-treated cells were heat shocked for 10 min before cross-linking and preparation of ChIP material. As shown in Fig. 6C, depletion of Paf1 markedly decreases the levels of trimethyl-H3-K4 observed at *Hsp70* after a 10-min heat shock. In fact, the levels of H3-K4 trimethylation detected in Paf1-depleted cells closely resemble the levels seen before heat shock, indicating that the methylation of promoter-proximal nucleosomes that occurs upon heat induction is dependent on the presence of Paf1.

DISCUSSION

Our characterization of the *Drosophila* homologs of yeast Paf1 subunits has revealed several features in common and several critical differences between the yeast and *Drosophila* Paf1 complexes. The most striking similarities between the yeast and *Drosophila* Paf1 complexes are their association with elongating RNA Pol II and their roles in gene activation, while

the nature of the Pol II association and the composition of the Paf1 complex reflect marked differences between the species.

The global view provided by *Drosophila* polytene chromosomes shows that the chromosome-associated Paf1 and Rtf1 proteins colocalize with active Pol II (Fig. 1A). This result supports the idea that these proteins participate in most, if not all, Pol II transcription. Remarkably, Paf1 and Rtf1 do appear to be separable from actively elongating Pol II under conditions of heat shock. Although Paf1 and Rtf1 are recruited actively to heat shock loci upon heat stress, these factors also remain associated with a number of additional sites on the chromosome, while Pol II is localized almost exclusively at heat shock loci under these conditions. These data suggest that Paf1 and Rtf1 may remain bound to the chromosome at activated genes through interactions with additional proteins.

It has been suggested that, in yeast, while the Paf1 complex is entirely nuclear in its localization (41), it has cellular functions that are independent of elongating Pol II (26, 33). The nucleolar association of Paf1 and Rtf1 that we observe on *Drosophila* polytene chromosomes could possibly represent such a function. At the nucleolar organizer (Fig. 1, NO), Paf1 shows broad labeling while the Rtf1 signal is restricted to the nucleolar periphery in a manner that is largely nonoverlapping. Interestingly, although the yeast Paf1 complex does not show strong nucleolar association normally, in an Rtf1 mutant, the Paf1 complex shows a strong association that is postulated to be a manifestation of its normal role in nuclear processing or export (33).

By using ChIP experiments, we obtained a higher-resolution view of the localization of Paf1, Rtf1, and Cdc73 at the *Hsp70* gene. The lack of a ChIP signal at *Hsp70* under uninduced conditions demonstrates that the presence of engaged Ser-5-P Pol II or the associated elongation factors such as Spt5 and TFIIS is not sufficient to recruit Paf1, Rtf1, or Cdc73 (Fig. 2B). Upon heat induction, we observe recruitment of all three proteins primarily within the coding regions of active *Drosophila* genes, rather than regions upstream of the promoter, or downstream of the site for cleavage and polyadenylation (Fig. 2C and D; see Fig. S3A in the supplemental material). The reduction in the Paf1 signal downstream of the polyadenylation site, which accompanies a decrease in the Pol II signal, likely signifies that Paf1 dissociates from chromatin within this region, consistent with recent results obtained with yeast (17, 19). However, we note that the absence of a significant Paf1 signal obtained with a given primer pair may simply indicate that the interactions of Paf1 with a particular region are transient.

The Paf1 complex in *S. cerevisiae* has been reported to be required for full Ser-2 phosphorylation of the Pol II CTD. This role of Paf1 in CTD phosphorylation regulation also appears consistent with the fact that *rtf1*Δ mutants show synthetic lethality with CTD kinase and phosphatase mutants in *CTK1* and *FCP1* (9). The lack of a Ser-2-P Pol II signal detected in yeast Paf1 mutants resulted in reduced recruitment of cleavage and polyadenylation factors, causing a defect in the polyadenylation of nascent transcripts (26). However, although depletion of *Drosophila* Paf1 or Rtf1 has a marked effect on induced *Hsp70* RNA levels, we saw no change in the levels of Ser2-P Pol II on the *Hsp70* gene in Paf1 or Rtf1 RNAi-treated cells (Fig. 5; see Fig. S4 and S5 in the supplemental material), indicating a difference between the functions of Paf1 in yeast and a metazoan system.

Another fundamental difference that we have observed between *Drosophila* and yeast Paf1 complexes is the relationship of the Paf1 and Rtf1 subunits in providing anchorage of the complex to Pol II. In yeast, Mueller et al. (26) have shown that the association of Paf1 with Pol II and active chromatin depends on the presence of Rtf1. In contrast, we find that the recruitment of Paf1 to activated *Drosophila Hsp70* is independent of Rtf1, while Rtf1 recruitment is dependent on Paf1 (Fig. 4B and C). These results may reflect the evolution of a more important role for the Paf1 protein in metazoans in providing affinity of the complex for Pol II, while Rtf1 became a more loosely bound component of the complex (35, 55, 56).

We have also investigated the role of *Drosophila* Paf1 in the modification of histones within actively transcribed regions. Whereas yeast Paf1 has been implicated in regulating the bulk levels of methylation of histone H3 at lysine residues 4 and 79 (27, 49, 50), we observe an effect of Paf1 depletion on the trimethylation of H3-K4, but not on di- or trimethylation of H3-K79 (Fig. 3A, bottom; see Fig. S5A in the supplemental material). Similarly, we observed that trimethylation of H3-K4 occurred within the promoter-proximal region of *Hsp70* and *Hsp26* upon heat shock and could be seen to increase from 2.5 to 10 min after heat induction, but we were unable to detect significant levels of H3-K79 dimethylation within the active *Hsp70* gene (Fig. 6B and C; data not shown). The latter result differs from results from other systems which link H3-K79 dimethylation with active transcription. However, it is consistent with recent data suggesting that both Grappa, the *Drosophila* H3-K79 methyltransferase, and the signal corresponding to H3-K79 dimethylation are localized to both active and intergenic regions of *Drosophila* polytene chromosomes (40; M. B. Ardehali, J. K. Werner, and J. T. Lis, data not shown). An alternative possibility is that the apparent differences between yeast and *Drosophila* result from the experimental systems used; RNAi treatments in *Drosophila* decrease, but do not completely abolish, their target, and thus the small amount of remaining protein may be sufficient to carry out certain functions. Conversely, the deletion mutants used to investigate yeast Paf1 entirely remove an important protein for many generations of cell growth, raising the possibility that some observed effects are indirect or secondary in nature.

It is interesting that although H3-K4 trimethylation depends upon Paf1 and the recruitment of Paf1 is temporally similar to H3-K4 methylation, the distribution of Paf1 appears to be spatially distinct from the promoter region where the strongest trimethylated H3-K4 signals are observed. Thus, our results suggest that the effects of Paf1 mutants on the modification of promoter-proximal nucleosomes (including the ubiquitination of H2B-K123) may occur through indirect mechanisms. These data are consistent with reports on yeast that indicate that the distribution of Paf1 subunits does not strictly correlate with the patterns of ubiquitinated H2B or methylated histone H3 (27). The localization of H3-K4 trimethylation reported herein is in agreement with the recently described distribution of Trithorax, a *Drosophila* H3-K4 methyltransferase (46). Furthermore, recent studies employing a *Drosophila* Trithorax mutant fly line suggest that a multiprotein complex that contains Trithorax plays a role in *Hsp70* gene activation (46). However, whether

the role of Trithorax in *Hsp70* activation is direct or indirect remains to be established. We note that we do not observe an effect of Paf1 depletion on the rates of Pol II recruitment, or distribution over the gene, suggesting that H3-K4 trimethylation may serve as a mark of transcription activation rather than a prerequisite for gene activation.

Our studies have provided new insights into the increased importance of the Paf1 complex in a metazoan system. It is significant that Paf1 is recruited in a manner that is spatially and temporally identical to that of chromatin-associated factors Spt6 and FACT. In agreement with the strong colocalization of Paf1 with these nucleosome-associated factors, we show that depletion of Paf1 significantly reduces the recruitment of both Spt6 and the FACT subunit SSRP1. A relationship among Paf1, Rtf1, and FACT is consistent with findings that an *rtf1*Δ mutation shows synthetic lethality with POB3, a subunit of the yeast FACT complex (9). Moreover, the FACT complex has been shown to interact with the Paf1 complex and the chromodomain-containing Chd1 protein at actively transcribed genes (44). In vitro, FACT has been shown to function optimally to facilitate transcription through nucleosomes when it is present at approximately one molecule of FACT per two nucleosomes; the effectiveness of FACT in promoting elongation is decreased dramatically below this threshold (30, 31). If these results reflect the situation in vivo, the greater than 50% decrease in FACT levels at the active *Hsp70* gene in Paf1-depleted cells would result in a rather pronounced effect on transcription through nucleosomes.

Furthermore, recent evidence obtained with yeast has shown that mutations of Spt6 or the FACT subunit Spt16 lead to aberrant chromatin architecture in the wake of elongating Pol II, presumably due to defects in reassembly of nucleosome structure (18). The failure to efficiently repackage transcribed DNA results in transcription initiation from cryptic sites and a reduction in levels of properly initiated and processed RNA. If a primary role of *Drosophila* Paf1 is to help stably recruit factors like Spt6 and FACT, then loss of Paf1 activity could also lead to the accumulation of nonfunctional or improperly processed RNA species. In support of this idea, a paper that was published during the preparation of this report states that mutations in yeast Spt6 alter the recruitment of Paf1 subunit Ctr9 and lead to defects in 3′-end processing of nascent RNA (17). It is thus tempting to speculate that the vast array of transcription elongation and RNA processing and export defects reported in yeast Paf1 mutant strains could result from perturbation of the nucleosome structure along actively transcribed genes. Moreover, it may be these chromatin and processing defects that account for the decrease in the amount of *Hsp70* mRNA that accumulates in response to heat shock in Paf1- or Rtf1-depleted cells.

Finally, the Paf1 gene in yeast is nonessential while the Paf1 gene in *Drosophila* is essential. This may reflect the more varied and demanding requirements of the transcription machinery in higher eukaryotes, where chromatin frequently plays a greater and more stringent role in regulation. This, in turn, may place a greater demand on the Paf1 complex, which appears to function at the interface between transcription and chromatin, perhaps serving as a platform that stimulates the association of a number of nucleosome-modifying complexes with actively elongating Pol II.

In summary, the gene for Paf1 is a required *Drosophila* gene that colocalizes with actively elongating Pol II when chromatin associated and plays a critical role in the activation of stress-induced genes. Furthermore, recent data reveal that mutations in parafibromin, the human homolog of the Paf1 complex subunit Cdc73, are associated with an elevated risk of parathyroid carcinomas; thus, the Paf1 complex may be a key regulator of cellular control in metazoans (35, 55). The connection between Paf1 and trimethylation of histone H3 at lysine 4 near the promoters of active genes is particularly interesting because a human homolog of Trithorax, the histone methyltransferase implicated in this activity, is ALL-1/MLL-1, which is associated with a number of acute leukemias (reviewed in reference 7). Future work to define the way in which Paf1 directs the histone methyltransferase activity of this key enzyme should provide insight into the interaction between active transcription and modifications of chromatin structure. Our data support a model in which the *Drosophila* Paf1 complex plays a key role in coordinating histone modifications and changes in nucleosome structure with transcription activation and Pol II elongation, thereby serving as a critical link between gene expression and chromatin structure.

ACKNOWLEDGMENTS

We thank Nick Fuda and Steve Mauro for critical reading of the manuscript and Sumusu Hirose for antibody to SSRP1. We also thank Ruth Seward and Dmitry Karachentsev of the Waksman Institute, Rutgers University, for help with preparation of *Drosophila* extracts.

K.A. was supported by National Research Service Award grant GM066661. This work was also supported by NIH grant GM25232 to J.T.L. and by the Howard Hughes Medical Institute and NIH grant GM37120 (to D.R.).

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