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The Human PAF1 Complex Acts in Chromatin Transcription Elongation Both Independently and Cooperatively with SII/TFIIS

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SUMMARY

Genetic and cell-based studies have implicated the PAF1 complex (PAF1C) in transcription-associated events, but there has been no evidence showing a direct role in facilitating transcription of a natural chromatin template. Here, we demonstrate an intrinsic ability of human PAF1C (hPAF1C) to facilitate activator (p53)- and histone acetyltransferase (p300)-dependent transcription elongation from a recombinant chromatin template in a biochemically defined RNA polymerase II transcription system. This represents a PAF1C function distinct from its established role in histone ubiquitylation and methylation. Importantly, we further demonstrate a strong synergy between hPAF1C and elongation factor SII/TFIIS and an underlying mechanism involving direct hPAF1C-SII interactions and cooperative binding to RNA polymerase II. Apart from a distinct PAF1C function, the present observations provide a molecular mechanism for the cooperative function of distinct transcription elongation factors in chromatin transcription.

INTRODUCTION

RNA polymerase II (Pol II)-mediated transcription is a highly coordinated process with three major steps (initiation, elongation and termination) that normally occur in the context of nucleosomal arrays within chromatin. Whereas nucleosomes may exert an inhibitory effect on transcription initiation, reflecting a block in preinitiation complex (PIC) formation (Knezetic and Luse 1986; Lorch et al., 1987; Workman and Roeder 1987), they also serve as a barrier to elongating Pol II complexes both in vitro (Bondarenko et al., 2006; Guermah et al., 2006) and in vivo (Han and Grunstein, 1988). During elongation in living cells, specific Pol II-associated factors help Pol II to overcome pausing resulting from specific DNA sequences, bound proteins or nucleosome structures (Li et al., 2007).

Genetic and biochemical approaches have identified several Pol II-associated transcription elongation factors that include SII/TFIIS, TFIIF, DSIF, Elongin, ELL, NELF, the PAF1 complex (PAF1C) and the histone chaperone FACT (Sims et al., 2004). Although transcription defects resulting from mutations in these factors are consistent with intracellular functions in transcription elongation, only a few such as SII (Kireeva et al., 2005; Guermah et al., 2006) and FACT (Orphanides et al., 1998) have actually been shown to have intrinsic transcription

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elongation activities on natural (chromatin) templates; and detailed mechanisms of action of many of these factors are just beginning to be understood (Sims et al., 2004). In addition, the presence of multiple elongation factors in eukaryotic cells suggests a potential for cooperative, as well as redundant, functions in chromatin transcription. In accordance with this, recent experiments have revealed genetic and physical interactions between distinct elongation factors (Arndt and Kane, 2003). However, there has been no direct biochemical evidence for cooperative functions in chromatin transcription.

PAF1C was originally identified in yeast as a Pol II-associated factor (Shi et al., 1996) comprised of the Ctr9, Leo1, Rtf1, Paf1 and Cdc73 subunits (Mueller and Jaehning, 2002). PAF1C is of special interest because it has been implicated in transcription elongation (Costa and Arndt, 2000; Betz et al., 2002; Squazzo et al., 2002; Rondon et al., 2004) and, potentially related, is required for H2B ubiquitylation and downstream H3K4 and H3K79 methylation both in yeast (Ng et al., 2003; Krogan et al., 2003; Wood et al., 2003) and in human (Zhu et al., 2005; Kim et al., 2009). However, there has been no direct biochemical evidence for a role in transcription elongation on chromatin templates independent of its role in histone modifications. Beyond demonstrated and presumed roles for PAF1C in histone modification and transcription elongation, respectively, genetic and biochemical studies in yeast have suggested PAF1C functions in other gene expression processes that include transcript site selection (Stolinski et al., 1997; Mueller and Jaehning, 2002) and mRNA processing steps that couple transcriptional and posttranscriptional events (Mueller et al., 2004; Sheldon et al., 2005; Rozenblatt-Rosen et al., 2009). Further emphasizing the importance of PAF1C in diverse cellular processes, but by as yet unknown mechanisms, recent studies in metazoans have identified PAF1C subunits as candidate oncogenes and tumor suppressors (Chaudhary et al., 2007) and established functions both in embryonic development and cell survival (Mosimann et al., 2006; Wang et al., 2008) and in the maintenance of ES cell identity (Ding et al., 2009).

Here we show that the human PAF1 complex (hPAF1C) exhibits not only an intrinsic transcription elongation activity that facilitates activator- and histone acetyltransferase-dependent transcription from a chromatin template but also, and perhaps more importantly, a strong cooperative function with SII. We also demonstrate a direct hPAF1C-Pol II interaction that is important for the intrinsic transcription activity of hPAF1C, as well as a direct interaction between hPAF1C and SII that results in cooperative binding to Pol II and is required for their synergistic function. These findings identify distinct hPAF1C functions and provide biochemical evidence for cooperative functions between distinct transcription elongation factors in activator-dependent transcription from chromatin templates.

RESULTS

Composition and Structural Organization of hPAF1C

Human PAF1C was affinity purified from a FLAG-hPAF1 HeLa cell line and found to contain homologues (hCTR9, hLEO1, hPAF1, hCDC73 and hRTF1) of the five yeast PAF1C subunits, as well as the SKI8 subunit unique to hPAF1C (Figure 1A). However, consistent with earlier reports of human and Drosophila PAF1Cs lacking RFT1 (Rozenblatt-Rosen et al., 2005; Yart et al., 2005; Zhu et al., 2005; Adelman et al., 2006), hRTF1 was at least partially dissociated from the native hPAF1C upon gel filtration (Figure 1B). Consistent with our demonstration of a 6-subunit complex in HeLa cells, we were able to reconstitute and affinity purify a similar complex, containing stoichiometric amounts of all 6-subunits, from Sf9 cells coinfected with baculoviruses that individually express the various subunits (Figures 1C and 1D). Given the pivotal role of the RTF1 subunit in PAF1C function in many organisms (Mueller and Jaehning, 2002; Ng et al., 2003; Adelman et al., 2006; Tenney et al., 2006; Warner et al., 2007; Ding et al., 2009), it is of interest that HeLa cells were found to contain several forms of hRTF1 that potentially arise from alternative splicing (Figure S1).

Taking advantage of the ability to selectively express recombinant hPAF1C subunits in insect cells, we determined the effect of individual subunit deletions on complex formation as well as direct interactions between pairs of co-expressed subunits. The results (detailed in Figure S2) led to establishment of the interaction network shown in Figure 1E. It is noteworthy, for functional and mechanistic studies described in later sections, that the extensive interaction network allows the reconstitution and isolation of complexes that effectively lack only one or two subunits. hPAF1 appears to be the key scaffold subunit since omission of any other subunit in the reconstitution of complexes purified through coexpressed FLAG-hPAF1 results in complexes lacking only that subunit (with the exception of concomitant hSKI8 loss when hCTR9 is deleted), whereas deletion of hPAF1 in the reconstitution of complexes purified through FLAG-hCTR9 or FLAG-hRTF1 may result in significantly diminished levels of an additional subunit (e.g., hLEO1 in the FLAG-hCTR9 complex and hCTR9 in the FLAG-hRTF1 complex; Figure S2). These results are consistent with genetic analyses in yeast indicating selective loss of PAF1C functions upon deletions of individual subunits (Mueller et al., 2004; Porter et al., 2005; Xiao et al., 2005), and also indicate that individual binary interactions are reinforced by more global cooperative interactions within hPAF1C.

An Intrinsic Effect of hPAF1C, As Well As a Synergistic Effect with SII, on p53- and p300-dependent Transcription from Chromatin Templates

In order to test for and to establish direct functions of transcription elongation factors on natural (chromatin) templates, we employed an in vitro transcription assay (schematized in Figure 2A) containing highly purified transcription factors (Pol II, TFIID, TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, PC4 and Mediator) and a chromatinized pML array template that contains p53-binding sites adjacent to a core promoter and generates 390-nucleotide transcription products. This system previously was shown to effect activator-dependent transcription both from a naked DNA template and, in conjunction with p300 and SII, from a chromatin template (Guermah et al., 2006).

Importantly, in testing for a possible effect of hPAF1C on p53- and p300-dependent transcription from a chromatin template, we observed dose-dependent stimulatory effects of both reconstituted (Figure 2B, lanes 7–10) and native (Figure 2C, lanes 1–4) hPAF1Cs, thus demonstrating an intrinsic ability of hPAF1C to enhance transcription from a chromatin template. Further analyses revealed that the hPAF1C effect is completely dependent upon p53 and p300/acetyl-CoA (Figure 2D) and cannot be overcome by saturating amounts of p300/acetyl-CoA (Figure S3A). These results establish non-redundant functions between p300 and hPAF1C.

The presence in living cells of multiple factors implicated in transcription elongation raises the possibility of their cooperative functions, as well as redundancies, in transcription. Having both a purified hPAF1C with recognized transcription activity on chromatin template and SII with its previously established function in transcription elongation (Guermah et al., 2006), we tested the possibility of synergism between these factors. Remarkably, the p53-dependent transcription activity observed with SII (in the presence of p300) was significantly enhanced by the reconstituted hPAF1C in a dose-dependent manner (Figure 2B, lanes 2–4), with the synergistic effect being saturated at the highest tested dose of hPAF1C (lane 5). The native hPAF1C also showed a comparable, albeit slightly lower, level of enhanced transcription relative to that obtained with the reconstituted hPAF1C (Figure 2C, compare lanes 8 and 10). However, the lower synergistic effect with the native complex relative to the reconstituted complex may simply reflect our inability to assay a comparable amount of native hPAF1C owing to its more dilute concentration. Of note, while the present study has focused on transcription events in response to p53, our demonstration of similar independent and

cooperative hPAF1C functions in NFκB(p65)-dependent transcription (Figure 2E) suggests that these functions will be broadly applicable to other activators.

In our purified transcription system, all general transcription factor preparations were titrated to determine the amounts that gave optimal activity. As an example of the verification of saturating levels of a factor, a two-fold increase in the normal Mediator concentration had no effect on the overall synergy between SII and hPAF1C (Figure S3B). This rules out the possibility that hPAF1C or SII is simply substituting for a limiting amount of a general transcription factor in the assay. In more detailed titrations of SII and hPAF1C (Figure 2F), hPAF1C, in a dose-dependent manner, markedly increased transcription at lower levels of SII (lanes 5–12) but only moderately increased transcription at the highest level of SII where the SII-dependent activity was higher (lanes 13–16). This is consistent with a mechanism, also supported by protein interaction studies (below), in which hPAF1C facilitates SII binding to Pol II for its subsequent (previously established) function in Pol II passage through nucleosomes.

Direct Evidence for Transcription Elongation Activities of hPAF1C and SII on a Chromatin Template

The results described thus far have not clearly addressed whether hPAF1C acts, either alone or synergistically with SII, during preinitiation complex (PIC) formation or during transcription elongation. To address this issue, we first assembled a complete PIC on a DNA template in the presence of p53 and purified transcription factors and then performed sequential chromatin assembly, p300-dependent histone acetylation, and transcription steps (Figure 3A). The results show that p300/acetyl-CoA and hPAF1C are both required at a step after PIC assembly for optimal activity in the absence of SII (Figure 3B, lane 4 versus lanes 2 and 3) and, similarly, that p300/acetyl-CoA and SII are both required at a step after PIC assembly for transcription in the absence of hPAF1C (lane 6 versus lanes 3 and 5). In addition, hPAF1C also exhibited strong synergy with SII in this assay (lane 7 versus lanes 4 and 6), thus suggesting that hPAF1C and SII work cooperatively at a postinitiation step.

To rule out the possibility in this analysis that hPAF1C or SII simply stabilizes and/or activates the PIC, even though they are added after PIC formation, and to provide more direct evidence for hPAF1C and SII function at the transcription elongation stage, we designed a new transcription elongation assay (Figure 3C) based on a combination and modification of earlier protocols (Izban and Luse, 1992; Renner et al., 2001; Ujvari and Luse, 2006). Since the generation of transcripts with at least 21-nuleotides is not strictly dependent upon transcription elongation factors in our chromatin-templated assay (Guermah et al., 2006), a short (17-nucleotide) radiolabeled transcript was first synthesized from the p53ML-17 chromatin template and, after purification of the early elongation complex, transcription then was allowed to proceed in the presence and in the absence of transcription elongation factors (Figure 3C, see Supplemental Experimental Procedures for details). Importantly, this procedure includes two critical purification steps that validate this assay. The first is gel filtration of assembled chromatin to remove free ATP and thus restrict synthesis in the next step to the short radiolabeled CpA-primed transcript. The second is gel filtration to separate the early elongation complex on the chromatin template from residual radiolabeled nucleotides so that all visualized elongating transcripts originate from preexisting radiolabeled short transcripts. The results show that SII (Figure 3D, lanes 5–7 versus lanes 2–4) and hPAF1C (lanes 8–10 versus lanes 2–4) can independently facilitate transcription elongation in a time-dependent manner. Remarkably, SII and hPAF1C also exhibited strong synergy in this assay (lanes 11–13 versus lanes 5–7 and lanes 8–10), with the most obvious effects at the shortest elongation times (lane 11 versus lanes 5 and 8). These data demonstrate unequivocally that hPAF1C has an intrinsic transcription activity and that it acts cooperatively with SII at a transcription elongation stage.

Direct Interactions of hPAF1C with Pol II and SII Are Required for Its Intrinsic and Synergistic Effects on Chromatin Transcription

To examine the role of individual PAF1C subunits in hPAF1C interactions and functions, and to relate functions to specific interactions, several different hPAF1 sub-complexes (containing FLAG-hCTR9) were reconstituted in insect cells and subsequently purified (Figure S4). Consistent with the hPAF1C subunit interaction network and the role of hPAF1 as a scaffold protein (above), purified complexes reconstituted in the absence of hLEO1, hPAF1, hRTF1 or both hLEO1 and hPAF1 lacked mainly these subunits but were recovered in lower amounts in the case of the hPAF1-deficient complexes (Figure S4).

Relevant to the mechanism underlying the intrinsic hPAF1C activity, and consistent with the original isolation of the yeast PAF1 complex in association with Pol II (Shi et al., 1996), purified hPAF1C was found to interact directly with purified Pol II (Figure 4A). Interestingly, whereas the hLEO1-deficient hPAF1C interacted normally with Pol II, hPAF1C lacking hPAF1 showed a significantly decreased binding compared to the intact complex and hPAF1C missing both hLEO1 and hPAF1 showed no detectable binding. These findings are consistent with the results of binding assays with individual purified hPAF1C subunits (Figure S4), which revealed that hPAF1 interacts strongly, and hLEO1 weakly, with endogenous Pol II (Figure 4B). In functional analyses, purified reconstituted complexes lacking either hLEO1 or hPAF1 showed only modest reductions in activity compared to the intact complex (Figure 4C, lanes 2–4) whereas the complex missing both hLEO1 and hPAF1 was completely inactive (lane 5). These results establish hPAF1 and hLEO1 functions, apparently partly redundant, in the intrinsic transcription activity of hPAF1C and further suggest that intact hPAF1C normally functions in this capacity mainly through a direct hPAF1-mediated interaction with Pol II.

Relevant to the mechanism underlying the observed functional synergy between hPAF1C and SII, purified hPAF1C showed a strong, direct interaction with a purified GST-SII fusion protein but not with GST (Figure 4D). Moreover, in a test for intracellular interactions, endogenous hPAF1C subunits were coimmunoprecipitated either with endogenous SII using an anti-SII antibody (Figure 4E) or with ectopically expressed FLAG-SII using M2 agarose (data not shown). These observations indicate that hPAF1C directly interacts with SII both in vitro and in vivo. In further analyses with individual purified hPAF1C subunits (Figure S4), hLEO1 and hPAF1, but not other subunits, were shown to interact directly and strongly with SII (Figure 4F). The hLEO1-and hPAF1-deficient complexes, but not the complex lacking both hLEO1 and hPAF1, retained the ability to bind to immobilized SII, in further support of roles for both hLEO1 and hPAF1 in hPAF1C-SII interaction (Figure 4G). Of note, since the isolated hPAF1 and hLEO1 subunits both bind strongly to SII, the lower level of binding of the hPAF1-deficent complex relative to that of the hLEO1-deficent complex appears to reflect a lower level of retained hLEO1 in the hPAF1-deficent complex (Figure S4).

In functional studies, the FLAG-hCTR9-derived hPAF1C, like the FLAG-hPAF1-derived hPAF1C, also showed a significant synergistic effect with SII in chromatin-templated transcription (Figure 4C, lane 8 versus lanes 2 and 7). Exclusion of either hLEO1 or hPAF1 led to about a 50% reduction in activity compared to that of the intact hPAF1C (lanes 9 and 10 versus lane 8), whereas the simultaneous omission of hPAF1 and hLEO1 reduced the transcription level to that observed with SII alone (lane 11 versus lane 7). Along with the binding data, these results clearly indicate that the synergistic effect of SII and hPAF1C is achieved by their physical interaction through the hLEO1 and hPAF1 subunits in hPAF1C. It is important to note, however, that the reduced transcriptional synergy of the hLEO1-deficient complex with SII (Figure 4C) contrasts with the normal strong interaction of this complex with SII (Figure 4G). This suggests, as shown below (Figure 5), that hLEO1 has a role in transcriptional synergy beyond the direct interaction with SII. Also of note, omission of hRTF1 from hPAF1C led only to a very modest decrease in the synergistic effect (Figure 4C, lane 12),

suggesting that hRTF1 is not a critical component for the synergistic function with SII but leaving open the possibility that it may contribute to other functions of hPAF1C (see Discussion).

Cooperative Binding of hPAF1C and SII to RNA Polymerase II

To elucidate the mechanism(s) underlying the synergism between hPAF1C and SII in chromatin transcription, we tested the possibility of cooperative binding of these factors to Pol II. As expected, endogenous Pol II in nuclear extract bound selectively to immobilized GST-SII relative to GST alone, but this binding was significantly enhanced by addition of purified hPAF1C (Figure 5A, lanes 1–5). Reciprocally, endogenous Pol II in nuclear extract bound selectively to an M2 agarose-immobilized (FLAG-hCTR9) hPAF1C relative to M2 agarose alone, and purified SII markedly enhanced this interaction (lanes 6–10). A similar analysis with purified Pol II revealed hPAF1C-mediated enhancement of Pol II binding to immobilized GST-SII relative to GST alone, indicating that the cooperative binding of hPAF1C and SII to Pol II is mediated by direct protein-protein interactions (Figure 5B).

Next, we tested whether the observed direct interaction between hPAF1C and SII is required for their cooperative binding to Pol II (Figure 5C). The strong hPAF1C-dependent binding of Pol II to SII (lane 4 versus lane 3) was greatly reduced by deletion of hLEO1 from hPAF1C (lane 6), more drastically reduced by deletion of hPAF1 (lane 7), and almost completely abolished by deletion of hPAF1 and hLEO1 (lane 5). In contrast, deletion of hRTF1 had no significant effect on the cooperative interactions (lane 8). It is important to note that whereas hLEO1 is required for the cooperative binding of hPAF1C and SII to Pol II, it is not required for the strong independent binding of hPAF1C to SII (Figure 4G). These results suggest that hLEO1 plays an especially important role, through its direct SII interaction, in the cooperative binding of hPAF1C and SII to Pol II. The fact that a solitary hLEO1 deletion has no effect on the SII-independent interaction of hPAF1C with Pol II (Figure 4A) and only a modest effect on the intrinsic hPAF1C transcription activity (Figure 4C) also argues both for a hLEO1-selective function and for the structural integrity of the hLEO1-deficient hPAF1C. Overall, these effects of hLEO1 and/or hPAF1 deletion on cooperative binding of hPAF1C and SII to Pol II correlate well with effects of the deletions on the functional synergy between hPAF1C and SII (Figure 4C), thus suggesting a causal relationship.

Recruitment of hPAF1C and SII to Endogenous Genes during Transcriptional Activation

In order to investigate in vivo functions of hPAF1C and SII during gene activation, the p53-dependent doxorubicin-inducible *p21* gene (Figure 6A) was subjected to comprehensive chromatin immunoprecipitation (ChIP) analyses using the amplicons summarized in Figure 6B. In agreement with past studies (Gomes et al., 2007), we observed (i) a basal level of p53 occupancy at p53 response elements (regions labeled “A” and “B” in Figure 6B) followed by doxorubicin-induced increases in p53 occupancy at these sites (Figure 6C), (ii) a significant basal level of Pol II (RPB1) occupancy at the promoter region (C) followed by doxorubicin-induced increases in Pol II occupancy both at the promoter (C) and along the coding regions (D, E, F) (Figure 6C), and (iii) doxorubicin-induced accumulation of histone modifications (acetylated and K4-methylated H3) associated with gene activation (Figure S5).

The SII occupancy pattern was similar to that observed for Pol II, implying that recruitment of SII to the chromatin is mainly dependent on Pol II, but with the exception that SII occupancy peaks at 6 h whereas Pol II occupancy continues to increase to at least 12 h (Figure 6C). ChIP analyses for several hPAF1C subunits showed patterns somewhat similar to that observed for SII, but with the following differences from SII and/or Pol II. First, association of hPAF1C subunits with chromatin showed time-dependent increases (up to 12 h) following induction at promoter and coding regions. Second, occupancy at the promoter region (C) was not significant

at the uninduced (0 h) stage, in contrast to the significant basal level occupancy by Pol II and SII, suggesting that recruitment of hPAF1C to the promoter region is tightly regulated by on-going transcription. Third, hPAF1C subunits showed higher levels of accumulation at coding regions (D, E, F) relative to the promoter region (C) than did SII, suggesting non-identical (but overlapping) mechanisms/roles for hPAF1C and SII in the transcription elongation process (see Discussion). Remarkably, hRTF1 showed a pattern of accumulation indistinguishable from those of other hPAF1C components, strongly indicating a role for hRTF1 in conjunction with other hPAF1C subunits.

Given the observed cooperative interaction of hPAF1C and SII with Pol II, we tested whether the distribution of SII on the active *p21* gene is dependent upon or modulated by hPAF1C. hPAF1 siRNA, but not a control siRNA, resulted in a significant and selective reduction of the cellular hPAF1 level relative to the (unaltered) levels of Pol II and SII (Figure 6D), as well as a concomitant decrease in the level of p21 transcripts (Figure 6E). As expected, the ChIP analysis (Figure 6F) revealed a near complete loss of hPAF1 occupancy on the *p21* gene in response to hPAF1 depletion. In contrast, but with the exception of a decreased occupancy in the promoter region (C) at the fully induced (12 h) stage that accords with the markedly decreased level of transcription at this time point (Figure 6E), hPAF1 depletion had essentially no effect on either the distribution or density of Pol II along the *p21* gene. This is consistent with results of similar studies in Drosophila (Adelman et al., 2006) and yeast (Mueller et al., 2004). Importantly, however, the hPAF1-deficient cells showed decreased levels of SII at the promoter (C) and early transcribing (D) regions, supporting our in vitro observation that hPAF1C enhances and/or stabilizes the interaction of SII with Pol II.

DISCUSSION

Although several factors have been implicated by genetic analyses in transcription elongation, only a few have been shown to act directly to facilitate transcription elongation of Pol II on natural (chromatin) templates. Here, our ability to reconstitute a recombinant hPAF1C and the availability of a biochemically defined chromatin-templated assay has allowed us to assess direct functions of hPAF1C. Our results show, first, that hPAF1C can act in conjunction with histone acetyltransferase p300, at the transcription elongation step, to facilitate activator-dependent transcription from chromatin templates and, second, that hPAF1C also functions cooperatively with SII in this process. Of special note, these results reflect activities distinct from the earlier described histone ubiquitylation and methylation-independent chromatin transcription activities of hPAF1C. From a mechanistic viewpoint, the intrinsic activity of hPAF1C was shown to depend on subunits that interact with Pol II and the synergistic activity with SII was shown to involve a direct interaction with SII that leads to cooperative binding of SII and hPAF1C to Pol II for facilitation of their intrinsic activities (Figure 7). By identification of additional, direct hPAF1C functions in transcription and by elaborating mechanistic aspects of both independent and cooperative functions with SII, our results expand our understanding of the increasingly complex transcription elongation process and present additional views both of individual elongation factor functions and of direct cross-talk between elongation factors.

Structural Organization of hPAF1C and Specific Subunit Functions

An analysis of intracellular interactions of combinations of hPAF1C subunits has led to a model, emphasizing a complex network of interactions, for the structural organization of hPAF1C (Figure 1E). hPAF1 and hCTR9 are shown to play key roles as scaffold proteins, in agreement with yPAF1 complex studies showing that loss of either yPAF1 or yCTR9 results in nearly identical severe phenotypes related to many different cellular processes (Betz et al.,

2002; Mueller and Jaehning, 2002). In addition, we also found that hCTR9 is essential for physical association of human-specific PAF1C subunit hSKI8 (Figure S2).

Although the five integral subunits of the yeast Paf1C are phylogenetically conserved, recent studies of human and Drosophila PAF1Cs failed to see an association of RTF1 (Rozenblatt-Rosen et al., 2005; Yart et al., 2005; Zhu et al., 2005; Adelman et al., 2006). However, our current demonstration of a metastable association of a short form of hRTF1 with the natural hPAF1C, as well as the ability to reconstitute a stable hRTF1-containing 6-subunit hPAF1C in insect cells, argue strongly for hRTF1 function within hPAF1C. This notion is further supported by colocalization of Drosophila (Adelman et al., 2006) and human (this study) RTF1 with other PAF1 subunits on active genes and by the demonstration that Drosophila (Adelman et al., 2006; Tenney et al., 2006) and human (J.K., and R.G.R., unpublished data) RTF1, like yeast RTF1, are critical for H2B ubiquitylation and H3K4 methylation. The loss of RTF1 causes relatively subtle phenotypes in yeast compared to the loss of other subunits such as PAF1 and CTR9 (Mueller and Jaehning, 2002), thus indicating more restricted functions for RTF1 as well as PAF1C functions beyond accessory roles in H2B ubiquitylation and H3 methylation. Consistent with these observations, and importantly, our demonstrated roles for hPAF1C in transcription from chromatin templates were found to be independent of hRTF1 but dependent on two subunits, hPAF1 and hLEO1, that interact directly with Pol II and SII. Importantly, however, whereas hLEO1 and hPAF1 are both critical for the cooperative binding of hPAF1C and SII to Pol II, only hPAF1 (and not hLEO1) is necessary for the SII-independent binding of hPAF1C to Pol II. Further emphasizing subunit-specific functions, LEO1 was earlier shown to be unimportant for H2B ubiquitylation and H3K4 methylation (Xiao et al., 2005). These observations indicate distinct roles for specific subunits in the multi-functional PAF1C (e.g., LEO1 for transcription; RTF1 for histone modifications; PAF1 for both).

In contrast to our identification of hPAF1 as the dominant Pol II-binding subunit, CDC73 was shown to directly interact with Pol II in yeast (Shi et al., 1997). Although this may simply reflect distinct subunit functions in different organisms, we do not exclude the possibility of a functional hCDC73-Pol II interaction in human cells. This is plausible since hPAF1C shows a much stronger interaction with Pol II than do individual hPAF1 or hLEO1 subunits (Figure 4), implying that cooperative interactions between hPAF1C subunits (including hCDC73) result in enhanced binding of the complex to Pol II.

Direct Functions of hPAF1C in Transcription Elongation

The importance of proving direct transcription elongation capabilities is that the presumptive functions in transcription elongation of factors identified by genetic studies may reflect indirect effects on transcription elongation. In the case of PAF1C, its presumed role in transcription elongation is based both on its occupancy of promoter and transcribed regions (Pokholok et al., 2002) and on a demonstrated role in H2B ubiquitylation and H3 methylation events that are dependent on ongoing transcription (Osley et al., 2004; Kim et al., 2005; Xiao et al., 2005). However, and especially in view of the diverse PAF1C functions indicated by genetic analyses (Introduction), it has been unclear whether PAF1C itself has a more direct function (s) in transcription elongation. Related to this important issue, the present study has clearly shown that hPAF1C has an activity -- an intrinsic ability to directly facilitate transcription elongation from chromatin templates -- that is clearly distinct from, and independent of, previously described indirect functions in histone modifications (Ng et al., 2003; Krogan et al., 2003; Wood et al., 2003; Zhu et al., 2005). This activity was further shown to be dependent upon an activator and p300 and to act during transcription elongation rather than PIC formation, consistent with the possibility that it might somehow be involved in nucleosomal disassembly and/or displacement in conjunction with p300-mediated histone acetylation, the ATP-dependent remodeling activity by ACF, and the histone chaperone activity of NAP1 (all of

which are present in the assay). Although hPAF1C alone shows a chromatin transcription activity that is reminiscent of that seen with SII alone, these two factors do exhibit important qualitative functional differences (M.G. and R.G.R., unpublished observations).

Of note, our current demonstration of a direct function of hPAF1C in transcription elongation and our recent demonstration of hPAF1C-dependent transcription-coupled H2B ubiquitylation involving a direct interaction between hPAF1 and hBRE1 complexes (Kim et al., 2009) also provide a mechanistic explanation for the role of PAF1C in H2B ubiquitylation. Thus, it is plausible that PAF1C-enhanced passage of Pol II through nucleosomes, whether directly or through enhanced SII recruitment (below), allows H2B ubiquitylation factors (recruited by PAF1C) easier access to the H2B ubiquitylation site. These results provide ultimate explanations for how PAF1C links transcription and H2B ubiquitylation machineries, why PAF1C is required for H2B ubiquitylation and why H2B ubiquitylation and H3K4/H3K79 methylation are concentrated on actively transcribing genes.

Cooperative Functions between Transcription Elongation Factors

Transcription by Pol II is much more efficient within intact cells, where various nucleosomal and higher order chromatin constraints are imposed, than in established in vitro transcription assays. This suggests a possible need for extensive synergy in vivo between transcription factors that might include cooperative functions of distinct transcription elongation factors. Supporting this idea, the present study provides a clear demonstration of synergy between two distinct elongation factors, hPAF1C and SII, in chromatin transcription. Toward an understanding of the underlying mechanism, we demonstrate, first, that the synergistic effect of hPAF1C is greater at sub-saturating levels of SII and, second, that there is a direct interaction between SII and hPAF1C (through the hPAF1 and hLEO1 subunits) that results in cooperative binding to Pol II and is required for their full synergistic function. The latter conclusion is strongly supported by the observation, mentioned above, that hLEO1, in contrast to hPAF1, is selectively required for the cooperative binding of hPAF1C and SII to Pol II and not for the SII-independent binding of hPAF1C to Pol II. Hence, one mechanism of action of hPAF1C, beyond that involved in SII-independent elongation (above), is to facilitate SII recruitment and/or stabilization for its previously established role in Pol II passage through nucleosomes (Kireeva et al., 2005; Guermah et al., 2006). The relevance of such a cooperative and possibly redundant mechanism is strengthened by the fact that simultaneous mutation of *PAF1* and *DST1* is lethal in yeast (Squazzo et al., 2002).

Despite their strong physical interaction and functional cooperativity in transcription elongation, hPAF1C and SII show non-identical (but overlapping) patterns of occupancy on the *p21* gene (Figure 6). This is entirely consistent with the likelihood that these transcription elongation factors have both distinct and coupled functions/mechanisms in transcription. As described below, PAF1C participates in multiple transcription-related processes (initiation, elongation, mRNA processing and histone modifications) that may involve differential localization and/or stabilization of PAF1C on regions extending from the promoter to the 3' end of gene. Hence, the overall PAF1C ChIP pattern represents the sum of the individual patterns for the different PAF1C functions, and any single strong process, such as the PAF1C-dependent H2B ubiquitylation that peaks with PAF1C subunits downstream of SII and Pol II (Kim et. al., 2009), may bias the distribution. Non-identical ChIP patterns are also evident for other interacting factors. Thus, despite established physical interactions of PAF1C with DSIF (Qiu et al., 2006) and FACT (Squazzo et al., 2002), PAF1C shows a ChIP pattern clearly distinct from those of DISF (Qiu et al., 2006) and FACT (cf. Figure 6 and Gomes et al., 2007). These considerations suggest that physical interactions and resulting cooperative functions of distinct transcription elongation factors do not necessitate their exact co-localization on actively transcribed target genes.

The presence of multiple elongation factors and their genetic and physical interactions strongly suggests a complex network of communications and cooperative functions between elongation factors during transcriptional control in eukaryotic cells. The present study, employing a defined assay system, sets the stage for subsequent studies of detailed functional and mechanistic aspects of transcription elongation factors, including their communication with gene-specific activators and components of the transcription and chromatin-modifying machineries. This is especially important for the PAF1 complex, with its expanding and, in some cases, gene-specific roles in various physiological processes (Introduction).

Possible Roles of hPAF1C at Distinct Stages of Transcription

Besides the PAF1C transcription elongation functions emphasized in this study, functions in other transcription-related processes (Introduction), including transcription initiation, have also been suggested. Thus, genetic studies in yeast have suggested key roles for PAF1C in TATA site selection (Stolinski et al., 1997; Mueller and Jaehning, 2002) and in communication with DNA-binding factors (Betz et al., 2002), whereas studies in metazoans have shown direct interactions with transcriptional activators (Mosimann et al., 2006; Youn et al., 2007). In further support of these observations, we also found that hPAF1C (i) interacts directly with p53 both in vitro and in vivo, (ii) increases transcription from histone-free DNA templates, where the elongation process in the purified system is not a major rate-limiting step and (iii) slightly stimulates the generation of short transcripts from a chromatin template (J.K. and R.G.R., unpublished data). These results indicate a possible role for hPAF1C at an initiation and/or an early post-initiation step in transcription and support the view that PAF1C is involved in multiple processes in transcriptional regulation. Related to this, the decreased transcription of specific genes that is observed in PAF1-deficient cells (Figure 6; Adelman et al., 2006; Ding et al., 2008) is potentially due to a combined effect of defects in several PAF1C functions in transcription.

EXPERIMENTAL PROCEDURES

Cell Lines, Nuclear Extracts and Native hPAF1C Purification

The FLAG-hPAF1 cell line was selected from HeLa S3 cells transfected with a FLAG-hPAF1-pCDNA3 plasmid. Derived nuclear extracts (Dignam et al., 1983), were incubated with M2 agarose in BC buffer (20 mM Tris [pH7.9], 0.2 mM EDTA, 20 % glycerol) containing 300 mM KCl (BC300; hereafter, the number indicates the KCl concentration added to the BC buffer) and 0.1% NP40 at 4°C for 4 hr. After extensive washing with BC300/0.1% NP40, bound hPAF1C was eluted with 0.3mg/ml FLAG peptide in BC200/0.02% NP40.

Reconstitution of hPAF1C

cDNAs for hCTR9 (Genebank accession number: BC058914), hLEO1 (BC018147), hPAF1 (AJ401156), hCDC73 (NM_024529), hRTF1 (BC015052) and hSKI8 (AK024754) were obtained from ATCC and subcloned into pFASTBAC1 vector with or without the N-terminal FLAG epitope. Corresponding baculoviruses were generated according to the manufacturer's instruction (Gibco-Invitrogen). Sf9 cells were infected with combinations of baculoviruses and proteins/complexes were affinity purified on M2 agarose (Kim et al., 2009).

Protein Interaction Assays

For assays with M2 agarose-immobilized proteins, reactions contained (i) reconstituted hPAF1Cs (5 μg for the intact complex) containing equivalent amounts of FLAG-hCTR9 and 750 ng purified native (untagged) Pol II (Figure 4A), (ii) 2 μg of individual purified FLAG-tagged hPAF1C subunits and 1 mg HeLa nuclear extract (Figure 4B) or (iii) 5 μg of purified FLAG-hPAF1C, 1 mg HeLa nuclear extract and 2 μg of purified SII (Figure 5A, right panel).

The general conditions for binding and washing were BC200/0.05%NP40. Bound proteins were analyzed by immunoblot with indicated antibodies. GST-pull down and coimmunoprecipitation assays were performed as described (Kim et al., 2005).

RT-PCR Assays

Total RNA was prepared using Trizol (Gibco-Invitrogen) and RT-PCR analyses were performed. Primers for conventional (Figure 6A) and quantitative (Figure 6E) PCR reactions are summarized in Table S1 and Table S2, respectively.

Chromatin Immunoprecipitation (ChIP) Assays

HCT116 cells grown in McCoy's 5A (Gibco-Invitrogen) were treated with 0.5 μM doxorubicin (Sigma) for the indicated times. ChIP analyses were performed according to the manufacturer's instructions (Upstate). Primers for quantitative PCR are summarized in Table S3. For the RNAi-coupled ChIP analyses in Figure 6F, HCT116 cells were treated for 48 hr with siRNA duplexes (Dharmacon) using oligofectamine (Gibco-Invitrogen) and then subjected to ChIP analyses as described above.

Chromatin Assembly and In Vitro Transcription

Detailed protocols are described in Supplemental Experimental Procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

Adelman K, Wei W, Ardehali MB, Werner J, Zhu B, Reinberg D, Lis JT. Drosophila Paf1 modulates chromatin structure at actively transcribed genes. Mol. Cell. Biol 2006;26:250–260. [PubMed: 16354696]

Arndt KM, Kane CM. Running with RNA polymerase: eukaryotic transcript elongation. Trends Genet 2003;19:543–550. [PubMed: 14550628]

Betz JL, Chang M, Washburn TM, Porter SE, Mueller CL, Jaehning JA. Phenotypic analysis of Paf1/Pol II complex mutations reveals connections to cell cycle regulation, protein synthesis, and lipid and nucleic acid metabolism. Mol. Genet. Genomics 2002;268:272–285. [PubMed: 12395202]

Bondarenko VA, Steele LM, Ujvari A, Gaykalova DA, Kulaeva OI, Polikanov YS, Luse DS, Studitsky VM. Nucleosomes can form a polar barrier to transcript elongation by Pol II. Mol. Cell 2006;24:469–479. [PubMed: 17081995]

Chaudhary K, Deb S, Moniaux N, Ponnusamy MP, Batra SK. Human RNA polymerase II-associated factor complex: dysregulation in cancer. Oncogene 2007;26:7499–7507. [PubMed: 17599057]

Costa PJ, Arndt KM. Synthetic lethal interactions suggest a role for the Saccharomyces cerevisiae Rtf1 protein in transcription elongation. Genetics 2000;156:535–547. [PubMed: 11014804]

Dignam JD, Lebovitz RM, Roeder RG. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 1983;11:1475–1489. [PubMed: 6828386]

Ding L, Paszkowski-Rogacz M, Nitzsche A, Slabicki MM, Heninger AK, de Vries I, Kittler R, Junqueira M, Shevchenko A, Schulz H, Hubner N, Doss MX, Sachinidis A, Hescheler J, Iacone R, Anastassiadis

K, Stewart AF, Pisabarro MT, Caldarelli A, Poser I, Theis M, Buchholz F. A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell 2009;4:403–415. [PubMed: 19345177]

Gomes NP, Bjerke G, Llorente B, Szostek SA, Emerson BM, Espinosa JM. Gene-specific requirement for P-TEFb activity and Pol II phosphorylation within the p53 transcriptional program. Genes Dev 2007;20:601–612. [PubMed: 16510875]

Guermah M, Palhan VB, Tackett AJ, Chait BT, Roeder RG. Synergistic functions of SII and p300 in productive activator-dependent transcription of chromatin templates. Cell 2006;125:275–286. [PubMed: 16630816]

Han M, Grunstein M. Nucleosome loss activates yeast downstream promoters in vivo. Cell 1988;55:1137–1145. [PubMed: 2849508]

Izban MG, Luse DS. The RNA polymerase II ternary complex cleaves the nascent transcript in a 3'→5' direction in the presence of elongation factor SII. Genes Dev 1992;6:1342–1356. [PubMed: 1378419]

Kim J, Guermah M, McGinty RK, Lee JS, Tang Z, Milne TA, Shilatifard A, Muir TW, Roeder RG. RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. Cell 2009;137:459–471. [PubMed: 19410543]

Kim J, Hake SB, Roeder RG. The human homolog of yeast BRE1 functions as a transcriptional coactivator through direct activator interactions. Mol. Cell 2005;20:759–770. [PubMed: 16337599]

Kireeva ML, Hancock B, Cremona GH, Walter W, Studitsky VM, Kashlev M. Nature of the nucleosomal barrier to RNA polymerase II. Mol. Cell 2005;18:97–108. [PubMed: 15808512]

Knezetic JA, Luse DS. The presence of nucleosomes on a DNA template prevents initiation by Pol II in vitro. Cell 1986;45:95–104. [PubMed: 3955658]

Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, Boateng MA, Dean K, Ryan OW, Golshani A, Johnston M, Greenblatt JF, Shilatifard A. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol. Cell 2003;11:721–729. [PubMed: 12667454]

Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128:707–719. [PubMed: 17320508]

Lorch Y, LaPointe JW, Kornberg RD. Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. Cell 1987;49:203–210. [PubMed: 3568125]

Mosimann C, Hausmann G, Basler K. Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo. Cell 2006;125:327–341. [PubMed: 16630820]

Mueller CL, Jaehning JA. CTR9, RTF1, and LEO1 are components of the PAF1/Pol II complex. Mol. Cell. Biol 2002;22:1971–1980. [PubMed: 11884586]

Mueller CL, Porter SE, Hoffman MG, Jaehning JA. The Paf1 complex has functions independent of actively transcribing Pol II. Mol. Cell 2004;14:447–456. [PubMed: 15149594]

Ng HH, Dole S, Struhl K. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. J. Biol. Chem 2003;278:33625–33628. [PubMed: 12876293]

Orphanides G, LeRoy G, Chang CH, Luse DS, Reinberg D. FACT, a factor that facilitates transcript elongation through nucleosomes. Cell 1998;92:105–116. [PubMed: 9489704]

Osley MA. H2B ubiquitylation: the end is in sight. Biochim. Biophys. Acta 2004;1677:74–78. [PubMed: 15020048]

Pokholok DK, Hannett NM, Young RA. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell 2002;9:799–809. [PubMed: 11983171]

Porter SE, Penheiter KL, Jaehning JA. Separation of the Saccharomyces cerevisiae Paf1 complex from RNA polymerase II results in changes in its subnuclear localization. Eukaryot. Cell 2005;4:209–220. [PubMed: 15643076]

Qiu H, Hu C, Wong CM, Hinnebusch AG. The Spt4p subunit of yeast DSIF stimulates association of the Paf1 complex with elongating RNA polymerase II. Mol. Cell. Biol 2006;26:3135–3148. [PubMed: 16581788]

Renner DB, Yamaguchi Y, Wada T, Handa H, Price DH. A highly purified RNA polymerase II elongation control system. J. Biol. Chem 2001;276:42601–42609. [PubMed: 11553615]

Rondon AG, Gallardo M, Garcia-Rubio M, Aguilera A. Molecular evidence indicating that the yeast PAF complex is required for transcription elongation. EMBO J 2004;5:47–53.

Rozenblatt-Rosen O, Hughes CM, Nannepaga SJ, Shanmugam KS, Copeland TD, Guszczynski T, Resau JH, Meyerson M. The parafibromin tumor suppressor protein is part of a human Paf1 complex. Mol. Cell. Biol 2005;25:612–620. [PubMed: 15632063]

Rozenblatt-Rosen O, Nagaike T, Francis JM, Kaneko S, Glatt KA, Hughes CM, LaFramboise T, Manley JL, Meyerson M. The tumor suppressor Cdc73 functionally associates with CPSF and CstF 3' mRNA processing factors. Proc. Natl. Acad. Sci. USA 2009;106:755–760. [PubMed: 19136632]

Sheldon KE, Mauger DM, Arndt KM. A Requirement for the Saccharomyces cerevisiae Paf1 complex in snoRNA 3' end formation. Mol. Cell 2005;20:225–236. [PubMed: 16246725]

Shi X, Chang M, Wolf AJ, Chang CH, Frazer-Abel AA, Wade PA, Burton ZF, Jaehning JA. Cdc73p and Paf1p are found in a novel RNA polymerase II-containing complex distinct from the Srbp-containing holoenzyme. Mol. Cell. Biol 1997;17:1160–1169. [PubMed: 9032243]

Shi X, Finkelstein A, Wolf AJ, Wade PA, Burton ZF, Jaehning JA. Paf1p, an RNA polymerase II-associated factor in Saccharomyces cerevisiae, may have both positive and negative roles in transcription. Mol. Cell. Biol 1996;16:669–676. [PubMed: 8552095]

Sims RJ 3rd, Belotserkovskaya R, Reinberg D. Elongation by Pol II: the short and long of it. Genes Dev 2004;18:2437–2468. [PubMed: 15489290]

Squazzo SL, Costa PJ, Lindstrom DL, Kumer KE, Simic R, Jennings JL, Link AJ, Arndt KM, Hartzog GA. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J 2002;21:1764–1774. [PubMed: 11927560]

Stolinski LA, Eisenmann DM, Arndt KM. Identification of RTF1, a novel gene important for TATA site selection by TATA box-binding protein in Saccharomyces cerevisiae. Mol. Cell. Biol 1997;17:4490–4500. [PubMed: 9234706]

Tenney K, Gerber M, Ilvarsonn A, Schneider J, Gause M, Dorsett D, Eissenberg JC, Shilatifard A. Drosophila Rtf1 functions in histone methylation, gene expression, and Notch signaling. Proc. Natl. Acad. Sci. USA 2006;103:11970–11974. [PubMed: 16882721]

Ujvári A, Luse DS. RNA emerging from the active site of RNA polymerase II interacts with the Rpb7 subunit. Nat. Struct. Mol. Biol 2006;13:49–54. [PubMed: 16327806]

Wang P, Bowl MR, Bender S, Peng J, Farber L, Chen J, Ali A, Zhang J, Alberts AS, Thakker RV, Shilatifard A, Williams BO, Teh BT. Parafibromin, a component of the human PAF complex, regulates growth factors and is required for embryonic development and survival in adult mice. Mol. Cell. Biol 2008;28:2930–2940. [PubMed: 18212049]

Warner MH, Roinick KL, Arndt KM. Rtf1 is a multifunctional component of the Paf1 complex that regulates gene expression by directing cotranscriptional histone modification. Mol. Cell. Biol 2007;27:6103–6115. [PubMed: 17576814]

Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J. Biol. Chem 2003;278:34739–34742. [PubMed: 12876294]

Workman JL, Roeder RG. Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. Cell 1987;51:613–622. [PubMed: 3677170]

Xiao T, Kao CF, Krogan NJ, Sun ZW, Greenblatt JF, Osley MA, Strahl BD. Histone H2B ubiquitylation is associated with elongating Pol II. Mol. Cell. Biol 2005;25:637–651. [PubMed: 15632065]

Yart A, Gstaiger M, Wirbelauer C, Pecnik M, Anastasiou D, Hess D, Krek W. The HRPT2 tumor suppressor gene product parafibromin associates with human PAF1 and Pol II. Mol. Cell. Biol 2005;25:5052–5060. [PubMed: 15923622]

Youn MY, Yoo HS, Kim MJ, Hwang SY, Choi Y, Desiderio SV, Yoo JY. hCTR9, a component of Paf1 complex, participates in the transcription of interleukin 6-responsive genes through regulation of STAT3-DNA interactions. J. Biol. Chem 2007;282:34727–34734. [PubMed: 17911113]

Zhu B, Mandal SS, Pham AD, Zheng Y, Erdjument-Bromage H, Batra SK, Tempst P, Reinberg D. The human PAF complex coordinates transcription with events downstream of RNA synthesis. Genes Dev 2005;19:1668–1673. [PubMed: 16024656]

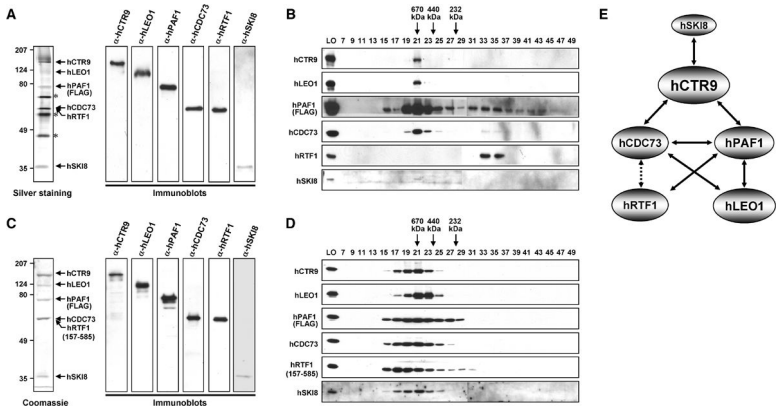

Figure 1. Characterization of Native and Reconstituted hPAF1Cs

(A) Silver staining and immunoblots of native hPAF1C purified from the FLAG-hPAF1 HeLa cell line. Non-specific proteins (marked by asterisks) are thought to be Hsp70, tubulin and actin (cf. Zhu et al., 2005). The indicated hRTF1 polypeptide is close in size to that encoded by residues 157–585 of the long hRTF1 open reading frame (Figure S1). (B) Immunoblots of purified native hPAF1C following Superose 6 gel filtration. Note that hRTF1 peaked separately from the circa 670 kDa complex and formed a stable trimeric complex with hPAF1 and hCDC73 through its C-terminus (Figure S1). (C) Coomassie blue staining and immunoblots of the purified, baculovirus-reconstituted hPAF1C. (D) Immunoblots of purified native baculovirus-reconstituted hPAF1C following Superose 6 gel filtration. (E) Multivalent hPAF1C subunit interactions. Arrows indicate direct interactions based on the analyses in Figure S2, with a weak interaction depicted by the dashed arrow.

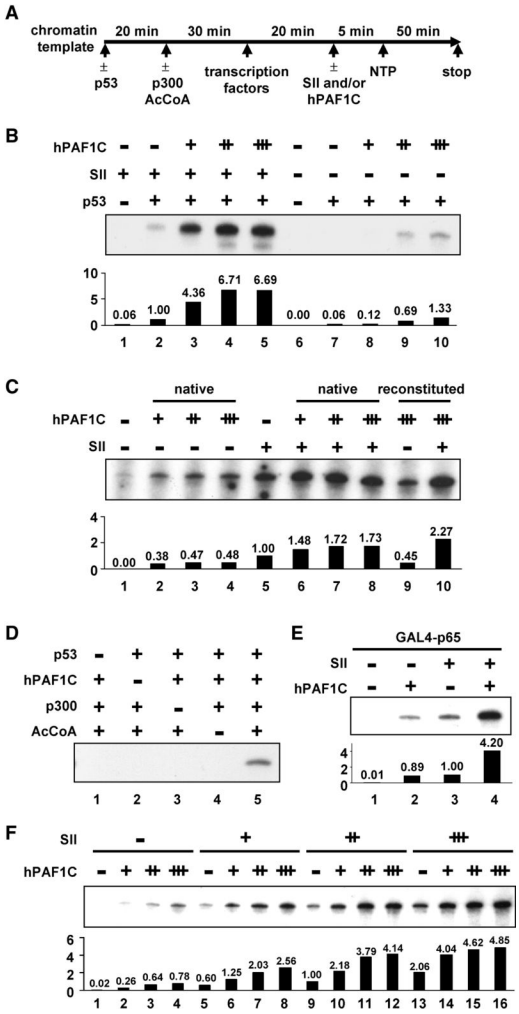

Figure 2. Synergistic Effect of hPAF1C and SII on p53-Dependent Chromatin Transcription

(A) Schematic representation of the standard in vitro transcription assay. Transcription factors included TFIIA, TFIIB, TFIIE, TFIID, TFIIF, TFIIH, PC4, Mediator and Pol II. Chromatin-based assays also contained the components (ACF1, ISWI and NAP1) employed for chromatin assembly. (B) Independent and cooperative effects (with SII) of reconstituted hPAF1C in chromatin transcription. Reactions contained p300/acetyl-CoA (all lanes) and, as indicated, 10 ng p53, 10 ng SII and either 160 ng (lanes 3 and 8), 320 ng (lanes 4 and 9) or 640 ng (lanes 5 and 10) of reconstituted hPAF1C. (C) Independent and cooperative effects (with SII) of native hPAF1C on chromatin transcription. Reactions contained p53 and p300/acetyl-CoA (all lanes) and, as indicated, 10 ng SII and either 17.5 ng (lanes 2 and 6), 35 ng (lanes 3 and 7) or 70 ng (lanes 4 and 8) of native hPAF1C or 70 ng (lanes 9 and 10) of reconstituted hPAF1C. (D) p53- and p300/acetyl-CoA- dependent function of hPAF1C. Reactions contained 10 ng p53, 15 ng p300 and 320 ng baculovirus-reconstituted hPAF1C, as indicated, but no SII. (E) Independent and cooperative effects (with SII) of hPAF1C in NFκB(p65)-dependent chromatin transcription. Reactions contained chromatin assembled with pG_5ML plasmid, 10 ng Gal4-p65 and p300/acetyl-CoA (all lanes) and, as indicated, 10 ng SII and 280 ng reconstituted hPAF1C. (F) Titration of hPAF1C and SII in an in vitro chromatin transcription assay. Reactions contained p53 and p300/acetyl-CoA (all lanes) and, as indicated, either 5 ng (lanes 5–8), 10 ng (lanes 9–12) or 20 ng (lanes 13–16) of SII and either 70 ng (lanes 2, 6, 10 and 14), 140 ng (lanes 3, 7, 11 and 15) or 280 ng (lanes 4, 8, 12 and 16) of reconstituted hPAF1C. In (B), (C),

(E) and (F), relative transcription levels were quantitated by phosphoimager and normalized to that of 10 ng SII alone. As shown in Figure S3, the effects of hPAF1C on transcription were still observed under saturating levels of Mediator and p300.

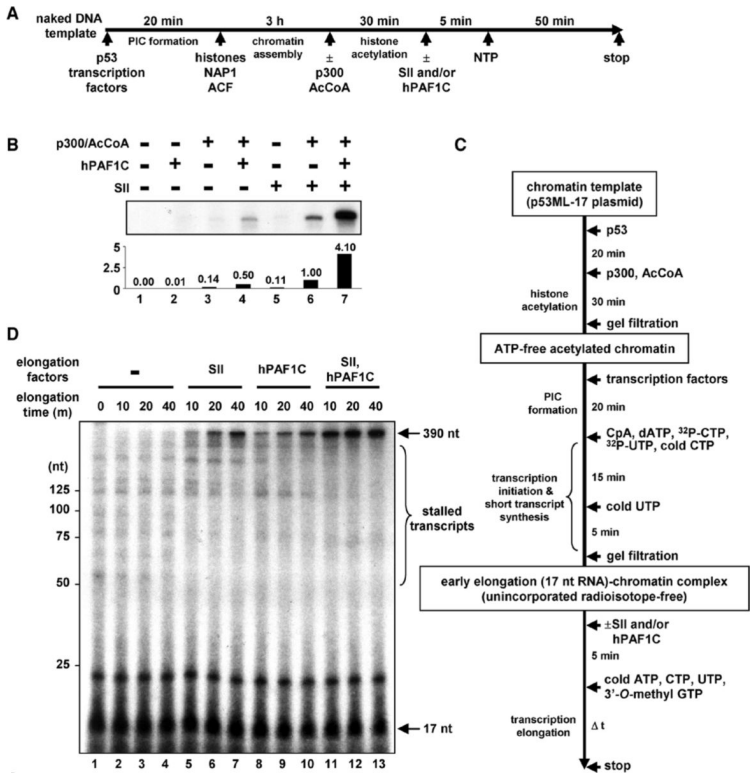

Figure 3. hPAF1C Independently and Cooperatively (with SII) Stimulates Transcription Elongation

(A) Schematic representation of the in vitro transcription assay in (B). (B) Independent and cooperative hPAF1C and SII functions after PIC assembly. Preinitiation complexes were formed prior to chromatin assembly by incubation of DNA with p53 and transcription factors. After subsequent chromatin assembly, sequential incubations with p300/acetyl-CoA, hPAF1C (320 ng), and SII (10 ng), as indicated, were carried out. Relative transcription levels were quantitated by phosphoimager and normalized to that of SII alone. (C) Schematic representation of the in vitro transcription elongation assay in (D). (D) Independent and cooperative hPAF1C and SII functions at the transcription elongation stage. Radiolabeled, chromatin-associated early elongation complexes were prepared according to the protocol in (C) and allowed to elongate in the presence of hPAF1C (320 ng) and/or SII (30 ng) as indicated.

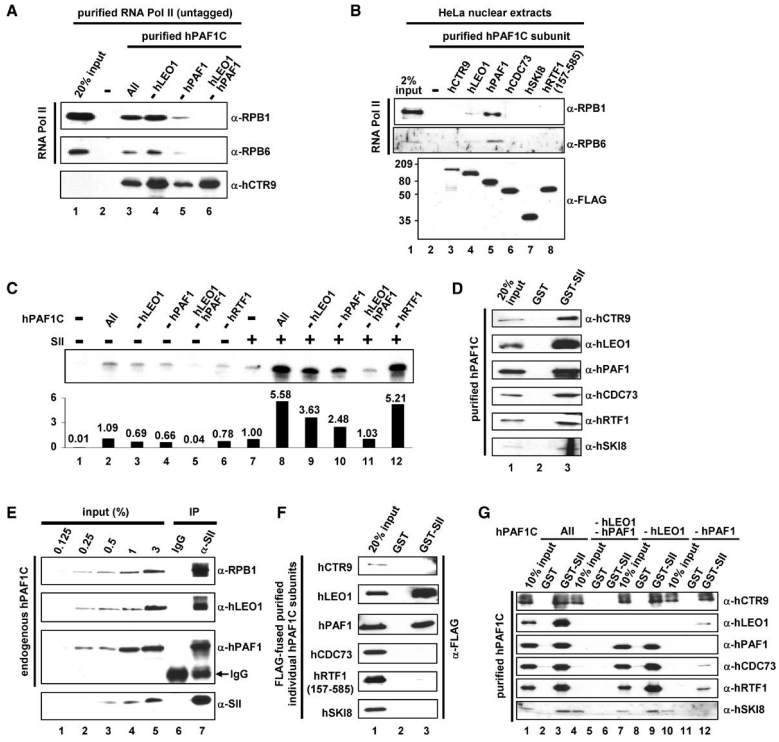

Figure 4. Direct Physical and Functional Interactions of hPAF1C with Pol II and SII

(A and B) Binding to Pol II of (A) purified hPAF1Cs that lack indicated subunits (Figure S4C) or (B) purified hPAF1C subunits (Figure S4B). (C) Chromatin transcription with SII and indicated hPAF1Cs. SII (10 ng) and reconstituted hPAF1Cs (320 ng for intact complex) containing equivalent amounts of FLAG-hCTR9 were assayed as indicated. p53 and p300/acetyl-CoA were added to all reactions. (D, F and G) Binding of (D) purified hPAF1C (Figure 1C), (F) purified hPAF1C subunits (Figure S4B) or (G) purified hPAF1Cs that lack indicated subunits (Figure S4C) to purified GST versus GST-SII (Figure S4A). (E) Intracellular binding of hPAF1C to SII. HeLa nuclear extracts were immunoprecipitated with anti-SII antibody and bound proteins were visualized by immunoblots with indicated antibodies (hRTF1 and hCDC73 are not shown because of their comigration with IgG heavy chain).

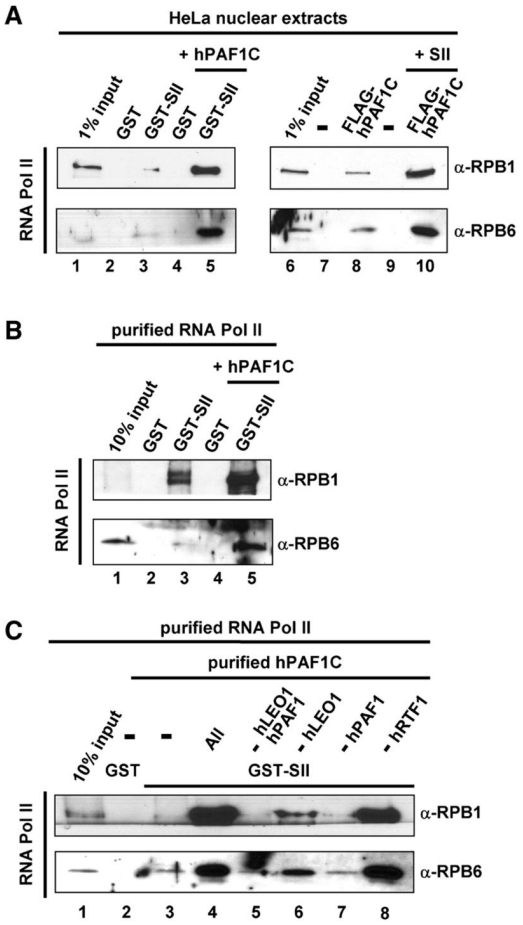

Figure 5. Cooperative Binding of hPAF1C and SII to Pol II

Binding of purified SII and hPAF1C to Pol II. HeLa nuclear extracts (A) or purified Pol II (B and C) were incubated with GST-SII or reconstituted hPAF1Cs (containing FLAG-hCTR9) in the presence and in the absence, respectively, of purified reconstituted hPAF1Cs or SII as indicated. hPAF1Cs in (C) were reconstituted with subunit omissions as indicated.

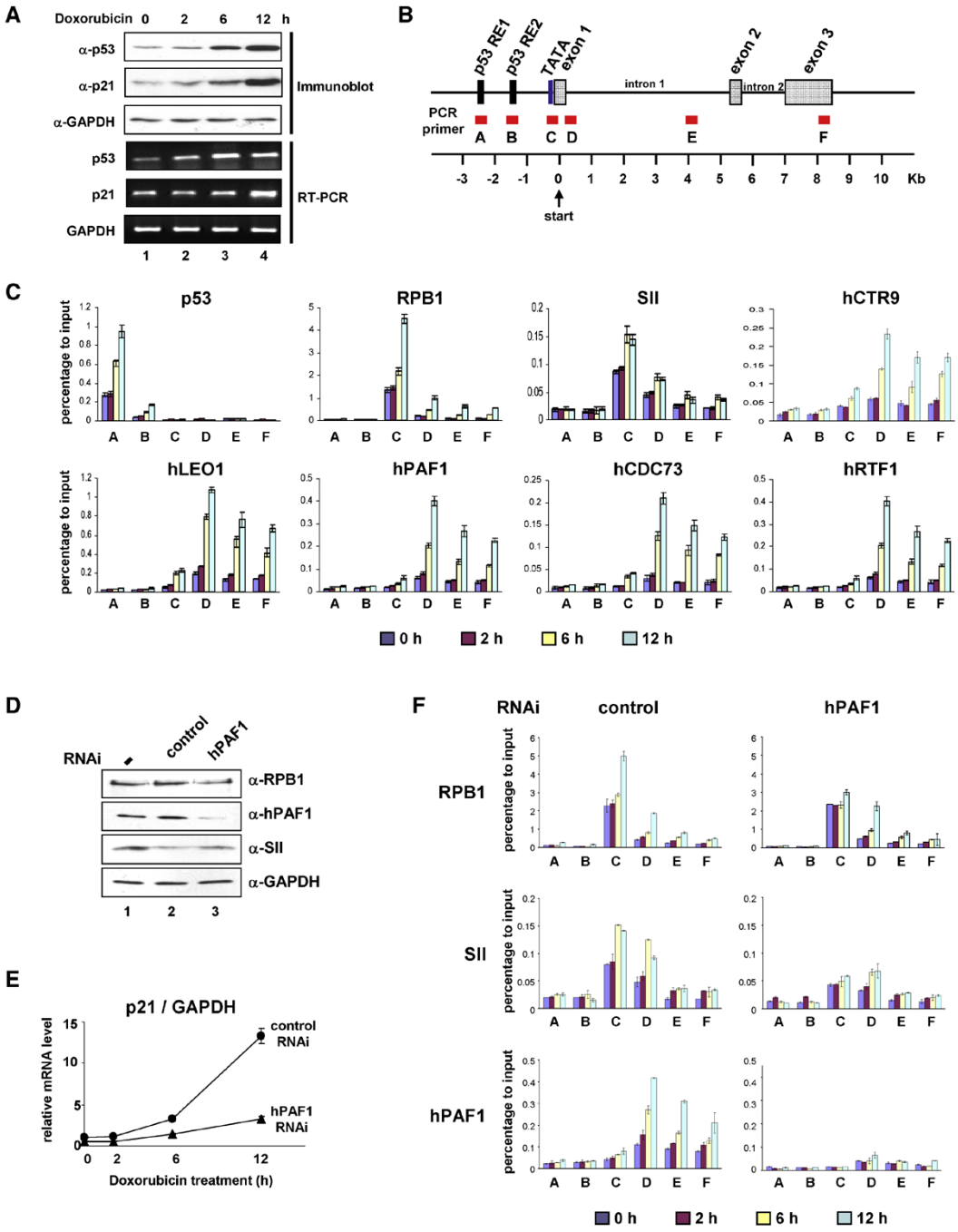

Figure 6. Distribution of hPAF1C and SII on the *p21* Locus during p53-Dependent Transcription

(A) Doxorubicin induction of p53 and the *p21* target gene. HCT116 cells were treated with 0.5 μM doxorubicin for the indicated times, and protein and mRNA levels were analyzed by immunoblot and RT-PCR, respectively. (B) Schematic representation of the *p21* locus indicating the six amplicons used for real-time PCR. (C) ChIP analyses on the *p21* locus. Cells were treated as in (A) and ChIP analyses were performed with indicated antibodies. Error bars denote standard deviations from three independent PCR reactions from a single ChIP that is representative of several that were performed. (D–F) HCT116 cells were treated with control and hPAF1-targeted siRNAs as indicated. Cell extracts were analyzed by immunoblots with indicated antibodies (D). p21 mRNA levels were measured by quantitative PCR analysis and

normalized to GAPDH mRNA. Error bars represent standard deviations from three independent PCR reactions (E). (F) After siRNA transfection, cells were treated with doxorubicin as in (A) and ChIP analyses were performed as in (C). [Note that the data in (C) are part of a comprehensive ChIP analysis and limited parts (e.g., p53 and p21 expression data and p53 and Pol II ChIP data) were recently published (Kim et al., 2009), for cross reference, with more extensive data on ubiquitylated H2B and H2B ubiquitylation factor analyses.] Additional data presented in Figure S5 document corresponding increases in H3 acetylation and H3K4 methylation on the *p21* locus during induction by doxorubicin.

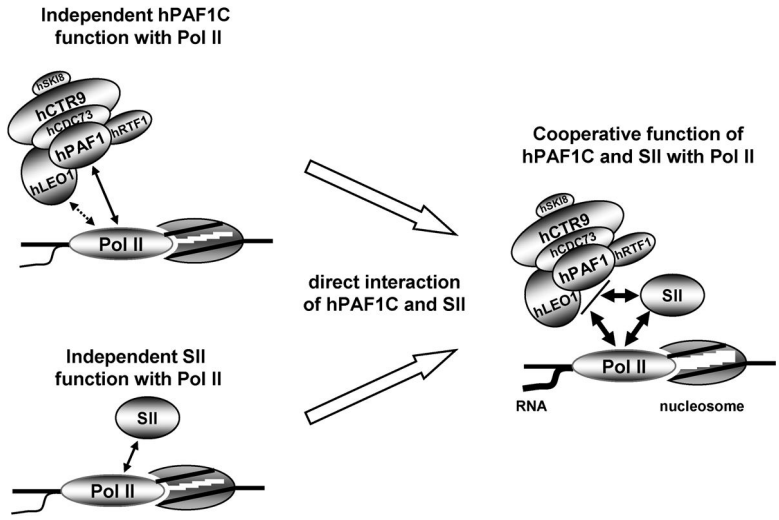

Figure 7. Models of Independent and Cooperative Functions of hPAF1C and SII in Transcription Elongation

Human PAF1C and SII independently facilitate transcription elongation through direct interactions with Pol II. In the case of hPAF1C, this involves a strong hPAF1-Pol II interaction (solid arrow) and a weak hLEO1-Pol II interaction (dashed arrow). While not shown, cooperative inter-subunit interactions within hPAF1C potentiate its interaction with Pol II relative to the independent interactions of individual subunits with Pol II. A direct interaction between SII and hPAF1C (via hPAF1 and hLEO1) results in their cooperative binding to Pol II (depicted by thick arrows) and a strong synergistic effect on transcription elongation.