Properties of PC4 and an RNA Polymerase II Complex in Directing Activated and Basal Transcription in Vitro*

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In eukaryotes, transcription of protein-encoding genes requires general transcription factors (GTFs) and RNA polymerase II (pol II), which are assembled on the promoter region to form a preinitiation complex (PIC) capable of producing RNAs by joining of TFIIB, pol II/TFIIF, TFIIE, and TFIIH, whereas in the two-component pathway, binding of TFIID is accompanied by a preassembled pol II complex that contains pol II, a subset of GTFs, SRBs (suppressor of RNA polymerase B mutations (5, 6)), and other proteins involved in chromatin remodeling, DNA repair, or mRNA processing (5–13). Transcriptional activators, in most cases, are able to increase the level of initiation by enhancing the recruitment of TFIID and/or other components of the basal transcription machinery to the promoter region (3, 4). This activation process often requires transcriptional coactivators. Thus far, two major classes of general coactivators for activator function have been identified in mammalian cell-free transcription systems. One is TBP-associated factors (TAFs) in TFIID (14–16), and the other is protein cofactors derived from the upstream stimulatory activity (USA) found in the phosphocellulose P11 0.85 M KCl fraction of HeLa nuclear extracts (17, 18).

Positive cofactor 4 (PC4) was isolated from a crude USA fraction and was able to substitute for USA to mediate activator-dependent transcription in vitro (19–21). PC4 is a nonspecific DNA-binding protein, which shows a higher affinity toward single-stranded (ss) DNA molecule (20–22). The ssDNA binding activity of PC4 can replace human ssDNA-binding protein (HSSB, also called replication protein A (RPA)) in supporting the T antigen-catalyzed unwinding of SV40 origin-containing duplex DNA (23). Nevertheless, PC4 cannot substitute for HSSB in other aspects of replication activities mediated by HSSB (23). Likewise, the transcriptional activity of PC4 cannot be replaced by other ssDNA-binding proteins (19). The coactivator function of PC4 seems to correlate with its double-stranded DNA binding activity (21) and its interactions with transcriptional activators and with components of the general transcription machinery such as TFIIF (19). Surprisingly, gene inactivation of a PC4 homologue in yeast does not lead to cell death, indicating that PC4 is nonessential in yeast (24, 25). Since yeast PC4 also exhibits distinct properties from that of human PC4 (24, 25), it is likely that PC4 may function differentially in various organisms. This remains to be further investigated.

Using an in vitro transcription system reconstituted with either TBP or TFIID and a preassembled pol II complex, we found that PC4 could function as a repressor to suppress basal transcription in the absence of an activator. Interestingly, TBP was able to mediate Gal4-VP16 activation in the absence of TAFs. This finding suggests that human TBP can indeed mediate activator function, as observed in the yeast system (26–28). To understand the molecular mechanism of PC4 repression, we carried out template challenge and Sarkosyl disruption experiments using our two-component transcription system. The results indicate that PC4 represses transcription by preventing the assembly of a functional preinitiation complex when an activator is not present.

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1 The abbreviations used are: GTF, general transcription factor; pol, polymerase; PC4, positive cofactor 4; SRB, suppressor of RNA polymerase B; TBP, TATA-binding protein; TAF, TBP-associated factor; TFIID, transcription factor IID; f:pol II, a FLAG-tagged RNA polymerase II complex; USA, upstream stimulatory activity; PIC, preinitiation complex; ss, single-stranded; HSSB, human single-stranded DNA-binding protein.
**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—A tetracycline-regulated human RPB9-expressing plasmid, pTetCMV-F-hRPB9, was first constructed by cloning the RPB9 cDNA (29), isolated from pBn-F-14.5 (provided by H. Ge) between NdeI and XbaI sites, into pTetCMV-F-AS (30) at the same enzyme-cutting sites. The expression plasmid, pRFIIIA (55-11d), was made by modifying the p55 insert from pET11aX-376 (31) into pET-RIIId (32) after removing the TBP insert between NdeI and EcoRI sites. Similarly, plasmids pRFIIIA (1-274)-11d, pRFIIIA (275-376), and pRFIIIF (hp12) were constructed by swapping individual TFIIF inserts from pJD1-274, pJDGEX2t (L275-376), and pGEKx2L (hp12) with the TBP insert from pTBP-11b between NdeI and BamHI sites.

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**Western Blotting**—To detect the presence of individual GTFs as described in the template challenge experiments. Ribonucleotide triphosphates were then added to initiate transcription. One hundred nanograms of PC4 were added at various time points as outlined at the bottom of Fig. 4.

**RESULTS**

**Isolation of a TFIIID-deficient Human Pol II Complex**—A human Pol II complex was purified from a clonal HeLa-derived cell line (hRPB9–3) that conditionally expresses the FLAG-tagged RPB9 subunit of human pol II (see “Experimental Procedures”). The purified FLAG-tagged pol II complex (f:pol II) contains not only pol II subunits as detected by Western blotting with antibodies against RPB1, RPB2, RPB6, RPB8, and RPB9 but also a subset of GTFs including TFIIB, TFIIE, TFIIF, and TFIIF (Fig. 1A). The f:pol II complex contains stoichiometric amounts of TFIIF and TFIIH but substoichiometric quantities of TFIIE and TFIIH (Fig. 1A, compare relative signals detected in nuclear extracts and f:pol II), as also evidenced by quantitative Western blotting using purified recombinant proteins as standards.3 TFIIID and TFIIH were not detected in f:pol II at a sensitivity of 1 ng with anti-TBP and anti-TAF1-55 antibodies and at a sensitivity of 0.1 ng with anti-TFIIA p35 antibodies (Fig. 1A).2 Our f:pol II, enriched approximately 200-fold after immunoaffinity purification, did not seem to contain other transcriptional cofactors such as PC4 and Dr1 and transcriptional activators including Sp1, YY1, USF, p53, pRB, and the p50 subunit of NFkB.2

**f:pol II Is Functional in a Highly Purified In Vitro Transcription System**—Recombinant human TFIIA, TFIB, TFIE, TFIF, and TFIIH were used in conjunction with either core-pol II (i.e., traditionally defined pol II) or immunoaffinity-purified f:pol II for transcriptional analysis. Both core-pol II and f:pol II, normalized by the content of RPB2 in the purified complexes, showed comparable levels of transcriptional activities irrespective of whether TBP or TFIIID was used as the TATA-binding factor (Fig. 1B, top and bottom panels, lanes 1 and 9). The pG5HMCAT template contains 5 Gal4-binding sites preceding the HIV-1 TATA box and the adenovirus major late (ML) initiator element in front of a 12493

1 S.-Y. Wu and C.-M. Chiang, unpublished data.

2 The f:pol II complex contains approximately 50 fmol µl−1 of pol II subunits, 70 fmol µl−1 of TFIIA, 135 fmol µl−1 of TFIIH, 1.2 fmol µl−1 of TFIIE, and 3.2 fmol µl−1 of TFIIH.
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pMLΔ53, which lacks the activator-binding sites, has a shorter G-less cassette (~280 nucleotides) driven only by the major late promoter TATA and initiator elements. In our transcription system, TFIIB, TFIIF, and a TATA binding activity (either TBP or TFIID) were essential for transcription by core-pol II (Fig. 1B, lanes 3, 4, and 6), whereas TFIIE and TFIIF, although not necessary for transcription from supercoiled DNA templates (44–46) (Fig. 1B, lanes 5 and 7), are required for transcription from linearized DNA molecules.2 Interestingly, transcription from different promoter elements seem to require differential amounts of TFIIE and TFIIF, as leaving out TFIIE and TFIIF affected transcription from pG,HMC2AT more dramatically than from pMLΔ53 (Fig. 1B, lanes 5 and 7). In contrast, transcription by f:pol II required only a TATA-binding factor (Fig. 1B, lanes 9–16), although leaving out TFIIE significantly reduced basal transcription from both DNA templates. The transcription data not only functionally confirm the identities of GTFs detected in our purified f:pol II (Fig. 1, A and B) but further suggest that a two-component pathway comprised of preassembled f:pol II and a TATA-binding factor is probably sufficient for the assembly of a functional preinitiation complex (see below). Obviously, TFIIF and TAFs were not needed for basal transcription from either DNA template (Fig. 1B, compare top and bottom panels, lanes 1 versus 2 and lanes 9 versus 10).

We also examined the factor requirement for activator-dependent transcription in our highly purified in vitro transcription system. All GTFs, except TFIIF, were necessary, in conjunction with a transcriptional activator (Gal4-VP16) and a coactivator (PC4), for activated transcription by core-pol II (Fig. 1C, lanes 1–10). In contrast, f:pol II only requires TFIID, PC4, and Gal4-VP16 for activated transcription from pG,HMC2AT (Fig. 1C, lanes 11–20). Again, leaving out TFIIE and TFIIF showed some effect on activated transcription by f:pol II, reflecting their substoichiometric amounts in purified f:pol II (Fig. 1A). Apparently, TFIIF was not required for activated transcription by either core-pol II or f:pol II in this highly purified in vitro transcription system (Fig. 1C, compare lanes 1 and 2 with lanes 11 and 12). Presumably TFIIF is only needed to antagonize the repressive effect from some negative factors that may be present in crude systems (2). Indeed, we have found that TFIIF becomes essential whenever a partially purified E/F/H or USA fraction is used in the transcription assay.4

In addition, the requirement for TFIIF can be affected by the amount of TFIIID and the promoter structure of the DNA templates used in the reaction (47). Nevertheless, this analysis indicates that, in addition to f:pol II and TFIIID, a transcriptional activator (Gal4-VP16) and a coactivator such as PC4 were minimally required for activated transcription in vitro.

**FIG. 1.** f:pol II is transcriptionally active in a highly purified in vitro transcription system. A, detection of general transcription factors in nuclear extracts and in the f:pol II complex. Western blotting was conducted as described under “Experimental Procedures” with a multiscreen apparatus (Bio-Rad) to divide samples into multiple lanes in which different antibodies were incubated individually with the same amount of protein samples in the lanes. Antibodies used were: normal rabbit sera (–), anti-TFIIF antibody (A), anti-TFIIB antibody (B), anti-TFIID TBP (T), and TAF\(_{74}\) (55) antibodies, anti-TFIIEx (α) and anti-TFIIExβ (β) antibodies, anti-TFIIF RAP30 (30) and RAP74 (74) antibodies, and anti-TFIIF p89 (89), p80 (80), and p62 (62) antibodies. The positions of some general transcription factors detected from nuclear extracts (upper panel) and f:pol II (lower panel) are indicated on the sides. The positions of prestained protein size markers (in kDa) are shown on the left. B, requirement of general transcription factors for basal transcription carried out by core-pol II or f:pol II in a highly purified in vitro transcription system. In vitro transcription was conducted as described under “Experimental Procedures” with recombinant TFIIF (A), recombinant TFIIB (B), recombinant TFIIE (E), recombinant TFIIF (F), FLAG-tagged TFIIF (HI), and either recombinant TBP (T; upper panel) or FLAG-tagged TFIIID (D; lower panel), in conjunction with either core-pol II (lane 1) or f:pol II (lane 9). The transcription components indicated above the lanes were then left out of the complete reaction. C, requirement of general transcription factors for activator-dependent transcription carried out by core-pol II and f:pol II. Transcription reactions were performed as described in B with the addition of PC4 and Gal4-VP16 (Act).
proteins used are: FLAG-tagged Gal4-Pro (various Gal4 fusion proteins as indicated. Recombinant Gal4 fusion (VP16 prised of f:pol II and either TBP (transcription was performed in a minimal transcription system com-

In vitro activation. binant TBP (under "Experimental Procedures" using f:pol II in conjunction with

FIG. 2. TAF-independent and TAF-dependent transcriptional activation. A, Gal4-VP16-mediated activation in a two-component transcription system. In vitro transcription was performed as described under "Experimental Procedures" using Epol II in conjunction with recombinant PC4 (rPC4), recombinant Gal4 (rGal4)-VP16, and recombinant TBP (T) or FLAG-tagged TFIID (D) as indicated. B, transcriptional activation mediated by various Gal4 fusion proteins. In vitro transcription was performed in a minimal transcription system comprised of Epol II and either TBP (lanes 1–7) or FLAG-tagged TFIID (lanes 8–14), in the presence (+) or absence (−) of recombinant PC4 and various Gal4 fusion proteins as indicated. Recombinant Gal4 fusion proteins used are: FLAG-tagged Gal4-Pro (Pro), FLAG-tagged Gal4-Gln (Gln), FLAG-tagged Gal4 (1–94), FLAG-tagged Gal4 (1–147), and Gal4-VP16 (VP16).

and 7). If Gal4-VP16 was added to the system without PC4, only minor if any enhancement of transcription was observed (Fig. 2A, compare lanes 2 and 3, and 7 and 8), confirming the importance of additional cofactors other than TAFs in mediating activator function (17, 36). Surprisingly, the coactivator PC4 in the absence of an activator acts as a repressor to suppress basal transcription mediated by TBP (Fig. 2A, compare lanes 2 and 4). PC4 repression was not obvious in the case of TFIID (Fig. 2A, compare lanes 7 and 9), indicating that TAFs can overcome PC4 repression. Surprisingly, when Gal4-VP16 was also provided, we observed transcriptional activation mediated by both TBP and TFIID (Fig. 2A, compare lanes 2 and 5, and 7 and 10). These data suggest that human TBP, in the absence of TFIID TAFs, can also mediate transcriptional activation in a mammalian cell-free transcription system, as previously shown in yeast (5). The presence of TAFs, however, help overcome PC4 repression and further enhance the level of activation mediated by TBP (Fig. 2A, compare lanes 7 and 9, and 7 and 10).

To see if TBP- and TFIID-mediated activation was unique to the acidic type of activation domains as exemplified by Gal4-VP16, we also tested the ability of other Gal4 fusion proteins in activating transcription in our minimal transcription system. Both Gal4-Pro and Gal4-Gln, which contain proline-rich and glutamine-rich activation domains linked, respectively, to the Gal4 DNA-binding domain, were able to activate transcription mediated by TBP, mainly at the level of antirepression (Fig. 2B, lanes 1–4). In contrast, Gal4(1–94), which was the portion used in Gal4-Pro and Gal4-Gln, had no effect on transcription (Fig. 2B, compare lanes 2 and 5). Interestingly, Gal4(1–147), which was used to make Gal4-VP16, was capable of activating transcription to a similar level as seen by Gal4-Pro and Gal4-Gln (Fig. 2B, lanes 1, 3, 4, and 7). This is consistent with the observation that an activation domain was present between amino acids 75 and 147 (19). This might explain why an additional level of transcriptional activation was observed with Gal4-VP16 in our minimal transcription system mediated by TBP (Fig. 2B, lanes 1 and 6); presumably two activation domains were synergistically used to activate transcription. When TAFs were present in our minimal transcription system, all these activators showed activation above the basal level (Fig. 2B, lanes 8–14). As mentioned earlier (Fig. 2A, lanes 2 and 4 versus lanes 7 and 9), PC4 did not repress basal transcription in the minimal transcription system when TFIID was substituted for TBP (Fig. 2B, lanes 1 and 2 versus lanes 8 and 9). We should emphasize that TAF-independent antirepression mediated by Gal4-Pro and Gal4-Gln was also an activation process, as it occurred only in the presence of an activator and only on the DNA template (i.e. pG5HMC2AT) containing the activator-binding sites.

PC4 Repression Could Also Be Alleviated by Preincubating TBP and fpol II with the DNA Template—Since PC4 is a nonspecific DNA-binding protein (20–22), it may inhibit transcription by blocking the access of protein factors to the promoter region. If so, PC4 repression should be alleviated by preincubating components of the general transcription machinery with the DNA template. To test this hypothesis, we first examined the requirement of protein factors for template commitment in our minimal transcription system reconstituted with TBP (or TFIID) and fpol II. Template commitment is usually the rate-limiting step for the assembly of a functional preinitiation complex (48) and is likely to be regulated by various transcription factors and cofactors. As outlined in Fig. 3A, both pG5HMC2AT and pMLΔ53 DNA templates were preincubated with TBP (or TFIID) or fpol II, individually or simultaneously, for 50 min. The other transcription components and ribonucleoside triphosphates were then added to initiate transcription. Reactions were continued for an hour before they were analyzed for RNA formation. In this experiment, a 10-fold excess of pG5HMC2AT template was added either during or after the preincubation period to test the stability of the protein-DNA complex. Demarcating the transcription reaction into two separate steps via order-of-addition did not change the overall yield of transcripts (Fig. 3, B and C, lanes 1 and 2). When excess pG5HMC2AT was added during the preincubation period, transcription from pMLΔ53 was reduced because less protein became available to the pMLΔ53 DNA template (Fig. 3, B and C, compare lanes 2 and 3, lanes 5 and 6, and lanes 8 and 9). If TFIID was preincubated with DNA templates before template challenge, no reduction of pMLΔ53 transcription was observed (Fig. 3B, compare lanes 2 and 4). This result was consistent with previous observations that TFIID could stably bind to the promoter region once committed (48–51). The same results were obtained by using pMLΔ53 as the challenge template. In contrast, preincubation of TBP or fpol II did not resist template challenge (Fig. 3B, compare lanes 5 and 7, and Fig. 3C, compare lanes 2 and 4, and lanes 5 and 7), indicating that TBP or fpol II alone could not stably bind to the promoter region. This result is also consistent with the observation that TBP, in the absence of other general transcription components, could not commit transcription to a particular template (51). Interestingly, when TBP and fpol II were both present during preincubation, this promoter-bound complex became stable and was thus resistant to template challenge (Fig. 3C, compare lanes 8 and 10). We conclude from these template challenge experiments that in our two-component transcription system only TFIID, but not TBP or fpol II alone, can stably bind to the
promoter region and thereby commit to the transcription process, consistent with previous results performed either with nuclear extracts or with complete transcription systems reconstituted with partially purified components (48–51).

We also examined if TBP or f:pol II would become stably bound to the promoter in the presence of a transcriptional activator and a coactivator. As shown in Fig. 3D, Gal4-VP16 and PC4 were unable to enhance the binding of TBP or f:pol II (lanes 6–8 and lanes 11–13). Only when both TBP and f:pol II were present would template commitment occur (Fig. 3D, lanes 16–18), consistent with the result performed in the absence of Gal4-VP16 and PC4 (Fig. 3C). Interestingly, preincubation of TBP and f:pol II together, but not individually, with PC4 and DNA templates alleviated PC4 repression (Fig. 3D, compare lanes 1 and 2, 4 and 5, 9 and 10, and lanes 14 and 15). This result suggests that PC4 may inhibit transcription via competition for binding to the promoter region, which is consistent with its role as a nonspecific DNA-binding protein. Once a stable preinitiation complex was formed on the promoter region, PC4 no longer displaced the stable complex.

**PC4 Inhibits the Assembly of a Functional Preinitiation Complex**—To further define the mechanism of PC4 repression, we divided the transcription reactions into multiple steps and performed an order-of-addition experiment with Sarkosyl challenge. In previous studies (52, 53), Sarkosyl was used to prevent the formation of a functional preinitiation complex. At a concentration of 0.015%, Sarkosyl would inhibit the formation of a preinitiation complex but not the preformed complex in transcription systems reconstituted either with partially purified GTFs (52) or with TBP and f:pol II.\(^2\) Therefore, only a single round of transcription should occur, if 0.015% Sarkosyl was added after formation of the preinitiation complex. Comparison of transcription signals obtained in the absence and presence of Sarkosyl (added at \(t_{0}\)) after preincubation, see the time course outlined at the bottom of Fig. 4) indicated that there are approximately two to three rounds of transcription in our two-component system (Fig. 4, compare lanes 3 and 7). If Sarkosyl was included during the preincubation period \((t_{0})\), transcription was abolished (Fig. 4, compare lanes 3 and 5) as no functional preinitiation complex could be formed in the presence of 0.015% Sarkosyl. We then asked if PC4 would also inhibit later steps of transcription, such as elongation and reinitiation, by adding PC4 at different time points after the assembly of the preinitiation complex. Consistent with the template challenge experiment (Fig. 3D), PC4 repression could be alleviated if transcription components were allowed to assemble on the promoter region before transcription started (Fig. 4, compare lanes 2, 4, and 10). However, the transcription level in the presence of PC4 was not restored to the original level without PC4 even after preincubation of TBP and f:pol II (Fig. 4, compare lanes 3 and 4, 9 and 10), indicating that PC4 might also affect elongation or reinitiation. When Sarkosyl was added to score for a single round of transcription, no inhibition by PC4 was observed (Fig. 4, compare lanes 11 and 12, lanes 15 and 16, lanes 19 and 20). These results suggest that PC4 also inhibits reinitiation but not elongation, in agreement with its role in preventing the assembly of a functional preinitiation complex.

**DISCUSSION**

The isolation of a TFIID-deficient f:pol II complex provides us with a unique opportunity to investigate the role of TAFs in the transcriptional process via a two-component transcription system reconstituted with f:pol II and either TBP or TFIID. The observations that TAFs indeed contribute to a high level of activation and that TBP can also mediate activation in our transcription system (Fig. 2) indicate that the previous discrepancy between in vivo yeast studies and in vitro mammalian cell-free transcription systems are not due to species variation. It is likely that some components of the general transcription machinery may affect TBP-mediated activation. Indeed we have recently observed that human TFIID has a significant effect on TBP-mediated activation,\(^4\) which can also be recapitulated in a transcription system reconstituted with individually purified general transcription factors. In addition to GTFs, our f:pol II also contains minor amounts of human SRBs such as cyclin C and CDK8, and some chromatin remodeling factors including GCN5 and BRG1,\(^7\) suggesting that f:pol II may correspond to previously characterized pol II holoenzymes (5–13). The exact relationship, however, remains to be further defined.
In our two-component transcription system, PC4 plays a dual role in the transcription process. In the absence of an activator, PC4 acts as a negative cofactor to suppress basal transcription, whereas in the presence of an activator, PC4 acts as a coactivator to mediate transcriptional activation. While this paper was under review, the Roeder laboratory (54) also reported that PC4 could inhibit basal transcription in a system reconstituted with only TBP, TFIIIB, TFIIIF, and pol II. Although the double-stranded DNA binding activity of PC4 has been shown to be essential for its coactivator function (21), it is not clear which PC4 activity is responsible for repression of basal transcription in the absence of an activator. Since deletion of the N-terminal 21 amino acid residues did not affect PC4 repression of basal transcription, it seems that the N-terminal SEAC (serine/acidic-rich) domain is not involved in transcriptional repression by PC4. It is therefore likely that PC4, being a nonspecific DNA-binding protein, competes with TBP or TFIIID for promoter binding. In the study of SV40 DNA replication, it was found that PC4 could inhibit the formation of RNA primers required for both leading and lagging strand DNA synthesis (23). This inhibition, however, could be partially reversed by the addition of excess HSSB (23), indicating that PC4 might repress RNA primer synthesis via its nonspecific DNA binding activity. The observations were consistent with our results that PC4 repression of basal transcription could be overcome by preincubation of transcriptional components with the DNA template (Figs. 3D and 4). Alternatively PC4 may interact with components of the basal transcription machinery, thereby titrating out critical protein factors needed for transcription. Indeed, we also observed that PC4 repression of basal transcription could be overcome by increasing amounts of TFIIID, TFIIH, or pol II in the transcription reactions. It is likely that activators may help stabilize or enhance the formation of a functional preinitiation complex, thereby overcoming repression by negative factors. The activation domain plays an essential role in this process, since the Gal4 DNA-binding domain alone cannot alleviate repression (Fig. 2B). Despite a low level of activator-dependent transcription, which was detected from pG5HMC2AT in the absence of PC4 in the minimal transcription system containing only Gal4-VP16, pol II, and TBP or TFIIID (Fig. 2A, compare lane 2 and 3, and lanes 7 and 8), the presence of PC4 significantly increased the overall level of activation (Fig. 2A, compare lanes 2, 3, and 5, and lanes 7, 8, and 10). It remains to be investigated if SRBs found in pol II may interact with specific cofactors that potentiate the activation mediated by Gal4-VP16.

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In this experiment, TBP and f:pol II were preincubated with DNA templates for 50.5 min (t0) before ribonucleoside triphosphates (NTPs) were added to initiate transcription. After a 60-min incubation, reactions were stopped and analyzed for RNA synthesis. PC4 was added at various time points (t1, t2, t3, or t4) during the reactions. Sarksy (0.015%), if added, was included either at the beginning (t0, labeled as “During”) or after 50 min (t5, labeled as “After”) of the preincubation period. Lanes 1 and 2 are the standard transcription reactions performed in parallel without the preincubation step. The presence (+) or absence (−) of PC4 and Sarksy in the reactions is indicated at the top of the gel.

FIG. 4. PC4 inhibits the formation of a functional preinitiation complex.

In the absence of an activator, PC4 acts as a negative cofactor to suppress basal transcription, whereas in the presence of an activator, PC4 acts as a coactivator to mediate transcriptional activation.
PC4 and TAF-independent Activation

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