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A dynamic model for PC4 coactivator function in RNA polymerase II transcription

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ABSTRACT Human positive cofactor (PC4) acts as a general coactivator for activator-dependent transcription by RNA polymerase II. Here we show that PC4 coactivator function, in contrast to basal (activator-independent) transcription, is dependent both on TATA binding protein (TBP)-associated factors (TAFs) in TFIID and on TFIIH. Surprisingly, PC4 strongly represses transcription initiation by minimal preinitiation complexes in the absence of TAFs and TFIIH, while simultaneously promoting the formation of these complexes. Furthermore, TFIIH and $TAF_{II}250$, the largest subunit of TFIID, can both phosphorylate PC4. These results provide evidence for an inactive, PC4-induced intermediate in preinitiation complex assembly and point to TFIIH and TAF requirements for its progression into a functional preinitiation complex. Thus PC4 coactivator activity is realized in a stepwise series of events reminiscent of prokaryotic activation pathways involving conversion of inactive RNA polymerase-promoter complexes to an initiation-competent state.

Activation of transcription of eukaryotic mRNA encoding genes by RNA polymerase II (pol II) involves three classes of transcription factors: general transcription factors (TFIIA, -B, -D, -E, -F, and -H), which act with pol II at core promoter elements to mediate specific initiation (1); activators, which typically function from upstream sites to transduce developmental and environmental signals to target genes; and coactivators, which operationally function to integrate the activities of general factors and activators. A set of general positive cofactors derived from the upstream stimulatory activity fraction (PC1, -2, -3, -4) and from other chromatographic fractions (PC5 and PC6) have been described in human cells (2), whereas studies in yeast have revealed an RNA pol II-associated complex of apparently distinct cofactors (3). Similarly, some TATA-binding protein (TBP)-associated factors (TAFs), which together with TBP constitute TFIID, are also thought to possess distinct types of coactivator properties (4, 5). Precisely how any of these coactivators fulfills its role remains unclear. However, because they interact with both a variety of activators and several components of the basal machinery, current models of activated transcription view them as providing an adaptor function that facilitates formation of a functional preinitiation complex (PIC) via recruitment of the general transcription machinery (1, 2, 5, 6).

PC4 was identified as a 15-kDa polypeptide that serves as a potent coactivator for a diverse group of activators in standard reconstituted *in vitro* transcription systems (7, 8). It interacts both with a variety of activation domains and with TFIIA (7), and its cofactor function strongly correlates with its ability to bind double-stranded DNA (9).

To decipher the mechanism by which PC4 stimulates transcription we have now employed a highly purified reconstituted *in vitro* transcription system to define the factors necessary for PC4 function. Our results reveal an intrinsic repressive property of PC4, possibly arising from its ability to facilitate early PIC assembly. However, repression can be reversed by combined effects of TAFs (in TFIID) and TFIIH, which are required for eliciting the full coactivator potential of PC4. Furthermore, and potentially related to this interconversion, PC4 can be phosphorylated within the PIC. Based on these observations, we propose a model for activated transcription which stresses that coactivator function in this process is not simply a matter of recruitment to the promoter but entails multiple steps.

MATERIALS AND METHODS

Purification of Transcription Factors. Basic chromatography procedures and buffer systems were essentially as described (10).

RNA pol II was purified from HeLa cell nuclear pellets by an adaptation of the method of Bitter (11).

Bacterially expressed histidine-tagged TBP was purified through sequential Ni-NTA-agarose and heparin-Sepharose steps (10). Histidine-tagged TFIIB (12) and the large (TFIIEα) and small (TFIIEβ) subunits of TFIIE (13) were purified on Ni-NTA-agarose. In some studies, a FLAG-tagged derivative of TFIIEα purified on M2-agarose was also used. Recombinant TFIIF was reconstituted from individually expressed His-tagged RAP74 and untagged RAP30 subunits as described (14). Bacterially expressed untagged PC4 was purified by heparin-Sepharose and phosphocellulose P11 chromatography (10). Natural TFIIA was isolated from the phosphocellulose P11 0.1 M fraction of HeLa cell nuclear extracts by further ion-exchange chromatography over DEAE cellulose (DE52) and an affinity matrix (Ni-NTA-agarose), as described (10). Recombinant TFIIA was reconstituted from bacterially expressed p12 and p55 (fusion protein precursor of subunits p35 and p19) (15).

Epitope-tagged TFIID (f:TFIID) was isolated from nuclear extracts from a cell line expressing FLAG-TBP (10). The phosphocellulose P11 0.85 M fraction was additionally purified on DE52. After binding and washing at 0.1 M KCl, the 0.15 M to 0.25 M KCl pool was subjected to M2-agarose affinity purification.

TFIIH was purified from the phosphocellulose P11 0.85 M fraction of HeLa cell nuclear extracts. After DE52 chromatography, where it coeluted with the general cofactor PC2 (16), TFIIH was further purified on MonoS, MonoQ, Superose 6 columns [fast protein liquid chromatography (FPLC); Pharmacia] and Ni-NTA-agarose.

Recombinant $TAF_{II}250$ was expressed in Sf9 cells via a baculovirus vector as a FLAG-tagged fusion protein. After

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Abbreviations: pol II, RNA polymerase II; TBP, TATA binding protein; TAF, TBP-associated factor; PC, positive cofactor; PIC, preinitiation complex; Ad ML, adenovirus major late; EMSA, electrophoretic mobility-shift assay; GST, glutathione *S*-transferase.

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affinity chromatography (M2-agarose), the protein was further purified over S-Sepharose, heparin-Sepharose, and S100-HR (Pharmacia).

***In Vitro* Assays.** Standard transcription and electrophoretic mobility-shift assay (EMSA) reactions were performed as described (10, 17, 18). Abortive initiation reactions (19–21) contained the indicated factors and appropriate nucleotides (including 10 μM dATP) in transcription buffer (10). After 1 hr at 30°C, calf intestinal phosphatase was added. Thirty minutes later reactions were terminated by addition of 10 mM EDTA and samples were directly loaded on 23% polyacrylamide gels containing 7 M urea.

RESULTS

PC4 Represses Transcription in the Absence of TAFs and TFIIH. A minimal system comprised of purified pol II and homogeneous preparations of recombinant general transcription factors TBP, TFIIB, and TFIIF mediates efficient basal transcription (22, 23) and provided a reference point for our analysis (Fig. 1*A*, lane 1). In this system, the use of a supercoiled pMLΔ53 template containing adenovirus 2 major late (Ad ML) core promoter elements obviated the TFIIH (and TFIIE) requirement for basal transcription (19, 21, 23). Under these conditions, amounts of PC4 that exhibited coactivator activity in the standard assay system potently repressed activator-independent (basal) transcription (Fig. 1*A*, lanes 1–3). Therefore, in the next series of experiments the minimal system was supplemented with the missing factor(s) to identify activities that allow(s) the bona fide coactivator function of PC4 to be manifested in a complete reconstituted system. Inclusion of recombinant TFIIE weakly stimulated basal transcription activity in this system but did not alleviate repression by PC4 (Fig. 1*A*, lanes 4 and 5). Similarly, TFIIA also did not overcome PC4 repression, either in the absence (Fig. 1*B*) or the presence (see below, Fig. 1*C*) of activator. This latter result is somewhat surprising because TFIIA interacts strongly with PC4 (ref. 7, and see below) and displays intrinsic coactivator and antirepression properties (15, 24).

Next, the effects of TFIIH, the TAF components of TFIID, and a GAL4-based transcriptional activator were assessed in reactions with a template (pG5ML) containing five GAL4 binding sites upstream of Ad ML core promoter elements (Fig. 1*C*). Reactions reconstituted with pol II and the basal factors TFIIA, TFIIB, TFIIE, and TFIIF were complemented either with TBP (lanes 1–4 and 9–12) or with an equivalent amount (normalized to TBP content by Western blot analysis) of homogeneous epitope-tagged TFIID (f:TFIID, ref. 10) (lanes 5–8 and 13–16). Basal transcription levels of TFIID-complemented reactions were severely reduced relative to those observed with an equimolar level of TBP alone (lane 1 vs. 5 and lane 9 vs. 13), because of both inhibitory effects of some TAFs on TBP function through TATA elements (25) and the absence in the minimal system of cofactors essential for synergism between Inr (initiator) and TATA elements (E. Martinez and R.G.R, unpublished results). Importantly, in the absence of TFIIH (lanes 1–8), the low basal activity in TFIID-containing reactions was also susceptible to inhibition by PC4 (lane 6 vs. lane 5). The addition of a limiting amount of TFIIH had only negligible effects on the levels of TBP- and TFIID-nucleated basal reactions in the absence of PC4 (lane 9 vs. lane 1; lane 13 vs. lane 5), consistent with the use of supercoiled templates in this assay. However, the same level of TFIIH afforded protection against inhibition of basal transcription by PC4 in a TFIID-nucleated reaction (lane 14 vs. lane 13) but not in a TBP-nucleated reaction (lane 10 vs. lane 9). Finally, in the presence of GAL4-AH, a strong activator that physically interacts with PC4 (7), the following was observed. First, no enhancement of TBP-nucleated transcription was evident; indeed, a slight reduction in transcription

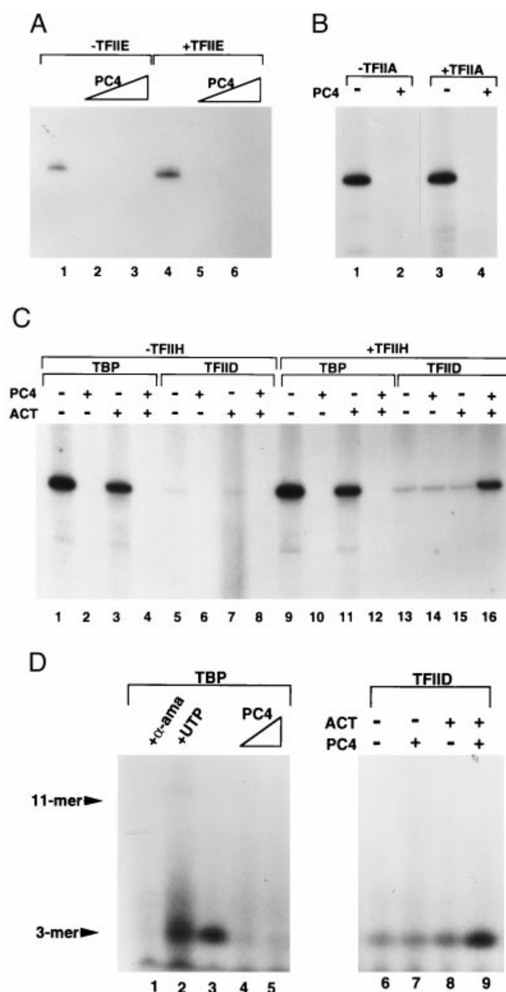

FIG. 1. Repression of transcription by PC4 in the absence of TAFs and TFIIH. (*A*) Minimal transcription reactions contained 2 ng TBP (6), 10 ng TFIIB, 25 ng TFIIF, 50 ng pol II, and 50 ng of pMLΔ53 template DNA (containing the Ad ML core promoter). Either 20 ng (lanes 2 and 5) or 100 ng (lanes 3 and 6) of recombinant PC4 and recombinant TFIIE (lanes 4–6), composed of 5 ng TFIIEα and 2.5 ng TFIIEβ subunits, were added as indicated. (*B*) Transcription reactions were assembled as in *A* and contained TFIIE. Reactions in lanes 3 and 4 were supplemented with a fraction containing TFIIA. A total of 75 ng PC4 was added as indicated. (*C*) Transcription reactions with the pG5ML template, containing five GAL4 sites upstream of the Ad ML core promoter were carried out as in *A* with either TBP (4 ng, lanes 1–4 and 9–12) or an equivalent (normalized to its TBP content by Western blot analysis) amount of f:TFIID (lanes 5–8 and 13–16). PC4 (75 ng) was added to reactions in each even-numbered lane and activator (GAL4-AH, 40 ng) to reactions in lanes 3, 4, 7, 8, 11, 12, 15, and 16. Reactions 9–16 contained purified (Ni-NTA-agarose fraction) TFIIH. All reactions contained TFIIA and TFIIE. (*D*) Abortive initiation reactions with the dinucleotide CpA and [α-^{32}P]CTP employed pG5ML as template. Reactions in lanes 1–5 were reconstituted with TBP, pol II, TFIIB, TFIIE, and TFIIF. Reactions in lanes 6–9 were reconstituted with f:TFIID, TFIIA, pol II, TFIIB, TFIIE, TFIIF, and TFIIH. PC4 (lanes 4, 5, 7, and 9) and GAL4-AH (lanes 8 and 9) were added as indicated.

levels in response to GAL4-AH was noted (lanes 1 vs. 3 and lanes 9 vs. 11), indicative of potentially nonproductive activator-general factor interactions in our minimal system. Second, and more to the point, GAL4-AH failed to reverse PC4-mediated repression of TBP-nucleated reactions in the presence or absence of a limiting amount of TFIIH (lane 4 vs. 3 and lane 12 vs. 11). Third, and in contrast to the preceeding results, in a TFIID-nucleated reaction, the activator elicited full coactivator activity of PC4 (with the absolute level of tran-

scription approaching that of the minimal TBP-nucleated reaction) in the presence (lane 16 vs. lane 15) but not in the absence (lane 8 vs. lane 7) of TFIIH. In other experiments (data not shown) with saturating amounts of TFIIH the activator-induced levels of transcription exceeded the basal levels of transcription obtained in the minimal system; however, under these conditions, some degree of protection against PC4 inhibition of the TBP-nucleated reaction was also observed.

To determine whether the PC4 restriction of minimal complexes was at the level of PIC formation or at a later step (e.g., initiation or elongation), we used an abortive initiation assay (19–21) to monitor the formation of the first phosphodiester bond (Fig. 1*D*). A specific trinucleotide product (CpApC) that corresponds to nucleotides at positions −1 to +2 of the Ad ML promoter start site was observed only in presence of the appropriate nucleotides (CpA and [^{32}P]CTP) and the minimal set of transcription factors (lane 3 and data not shown). The abortive initiation product was sensitive to α-amanitin (lane 1), and chased into longer RNA chains upon addition of one (lane 2) or both (data not shown) of the missing nucleotides. Addition of PC4 to TBP-nucleated minimal complexes completely blocked synthesis of the abortive initiation product (lanes 4 and 5), confirming that the PC4-mediated restriction to transcription occurs prior to the synthesis of the first phosphdiester bond (initiation). By contrast, but mirroring the results of Fig. 1*C*, PC4 did not suppress abortive initiation from a complete transcription system containing TFIID in place of TBP (Fig. 1*D*, lanes 6–9). Indeed, in the presence of the activator, PC4 led to elevated levels of abortive product synthesis from a template containing cognate sites for the activator (lane 9 vs. lane 8). Control experiments confirmed that the coactivator- and activator-dependent stimulation is specific because it was not observed with a template lacking cognate sites for the activator (data not shown).

These results argue strongly for a joint role for TAF and TFIIH components in normal PC4 function, and further imply that these distinct classes of transcription factors functionally synergize for maximal transcription activation. From a mechanistic standpoint, these data indicate that without TAFs and TFIIH, PC4 interactions with the basal transcription apparatus lead to loss of function.

PC4 Does Not Interfere with Minimal PIC Assembly. In a further analysis, EMSA was employed to identify the step at which PC4 inactivates minimal PICs. An oligonucleotide containing Ad ML promoter sequences from nucleotide positions −40 to +10 relative to the transcription start site was used as a probe to monitor effects of PC4 on formation of promoter subcomplexes by TBP, TFIIB, TFIIF, and pol II (Fig. 2*A*). Under the conditions selected, complex formation by TBP was not readily evident (lane 1), although a weak TBP-TFIIB (T·B) complex could be observed in the presence of TFIIB (lane 2). Further addition of pol II and TFIIF led to efficient formation of the expected higher order complex (T·B·F·II) but with the resulting EMSA band being somewhat diffuse (lane 4). Consistent with the deduced order of entry of purifed factors into the PIC (1), the appearance of this complex was dependent upon TFIIF as well as TBP and TFIIB (lanes 5, and data not shown). As reported previously (5, 6), there was no apparent effect of PC4 on TBP binding (lane 1 vs. 11). In marked contrast, T·B complex formation was dramatically stimulated by PC4 (Fig. 2*A*, lane 2 vs. lane 12). Moreover, the presence of PC4 led to a better defined higher order T·B·F·II complex that migrated slightly faster (lane 4 vs. lane 14). This finding may reflect an altered pol II conformation in the PIC (e.g., through an interaction with PC4, see below) or PC4-facilitated compaction of the template DNA. Inclusion of TFIIA in binding reactions yielded discrete T·A and T·A·B complexes (lanes 6 and 7), consistent with previous observations, but had no significant effect on higher order complexes (lane 9 vs. lane 4 and lane 19 vs. lane 14). Furthermore, as reported (7, 8), PC4 supershifted the T·A and T·A·B complexes formed in the presence of TFIIA, without substantial effect on the overall

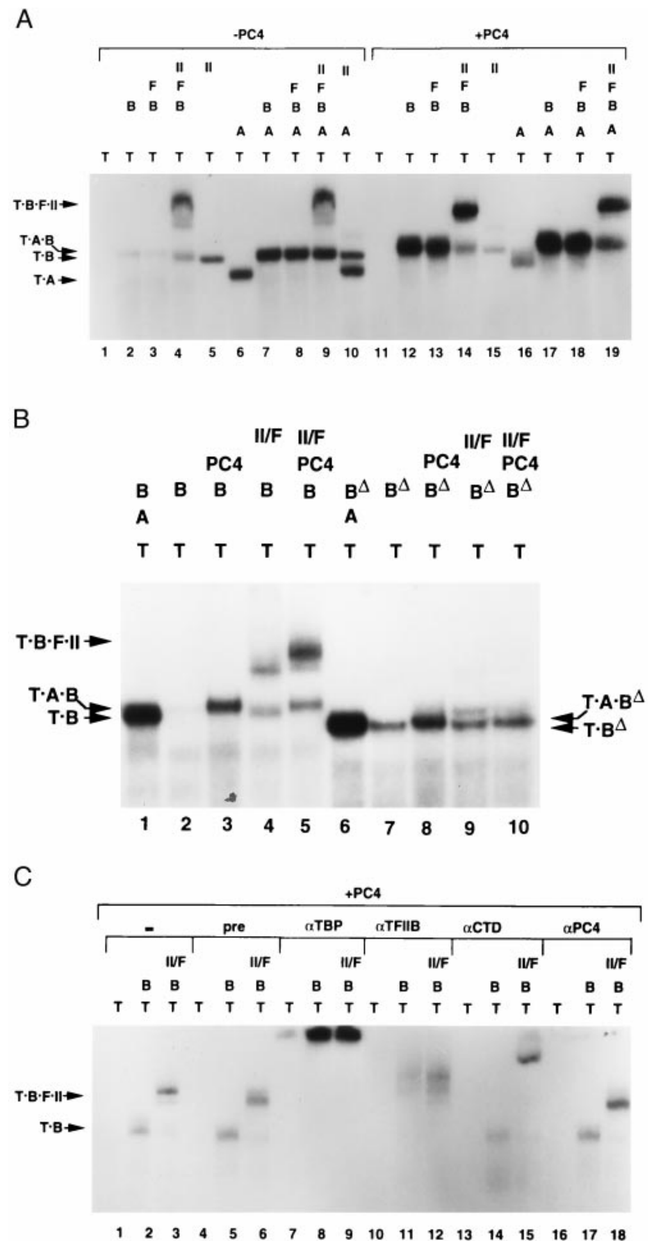

FIG. 2. PC4 does not interfere with formation of TBP-mediated PICs. (*A*) For EMSA, an end-labeled Ad ML oligonucleotide (−40 to +10) probe was incubated with the indicated combinations of factors: TFIIB (B, 20 ng, lanes 2–4, 7–9, 12–14, and 17–19); TFIIF (F, 25 ng, lanes 3, 4, 8, 9, 13, 14, 18, and 19); pol II (II, lanes 4, 5, 9, 10, 14, 15, and 19). Reactions were carried out in the absence (lanes 1–5 and 11–15) or presence (lanes 6–10 and 16–19) of native TFIIA (A). PC4 (75 ng) was added in lanes 11–19. All reactions contained TBP (0.5 ng). In the absence of TFIIB and TFIIF, pol II yields a nonspecific band (lanes 5, 10, and 15). Reactions were incubated at 30°C for 30 min and resolved by native PAGE. Free probe has been excised from the autoradiograms. (*B*) EMSA reactions were performed as in *A* with either wild-type (lanes 1–5) or N-terminal truncated TFIIB (ΔB, lanes 6–10). TFIIA was added to reactions 1 and 6; pol II and TFIIF (II/F) to reactions 4, 5, 9, and 10; and PC4 to reactions 3, 5, 8, and 10. All reactions contained TBP. (*C*) EMSA reactions contained TBP and PC4 and, as indicated, TFIIB (lanes 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, and 18) and pol II plus TFIIF (lanes 3, 6, 9, 12, 15, and 18). Preimmune (pre, lanes 4–6), anti-TBP (lanes 7–9), anti-TFIIB (lanes 10–12), anti-CTD (pol II, lanes 13–15), and anti-PC4 antisera were added after 10 min of incubation at 30°C.

extent of complex formation (Fig. 2*A*, lane 16 vs. lane 6 and lane 17 vs. lane 7).

Additional control experiments showed that T·B complex formation by core TFIIB (B^{Δ}) was also facilitated by PC4 (Fig. 2*B*, lanes 7 and 8). But because core TFIIB lacks the amino-terminal region implicated in pol II interactions (26), it did not support pol II recruitment into the complex regardless of the presence or absence of PC4 (lane 10 vs. lane 5 and lane 9 vs. lane 4). This result confirmed that PC4 preferentially acted on specifically assembled (TFIIB-dependent) pol II complexes. The identity of the various PC4-dependent complexes was further verified by antibody supershift analyses, which provided clear evidence for the presence of TBP, TFIIB, and pol II in the appropriate complexes but, unexpectedly, not that of PC4 (Fig. 2*C*). This result may be attributed to inaccessability of the epitopes on this rather small polypeptide or, alternatively, to an inability of PC4 containing complexes to survive electrophoresis (see below, Fig. 4*C*). We conclude that unlike conventional repressors such as NC2/Dr1·DRAP1 and MOT1 (27–29), PC4 does not inactivate (TAF-deficient) transcription complexes by interfering with PIC assembly.

PC4 Interacts with Multiple Components of the PIC. In view of these results we used immobilized GST-PC4 to test for direct physical interactions of PC4 with TFIIB and pol II (Fig. 3). As controls, TBP and recombinant TFIIA were also included in our analysis. In accord with published results (7), TFIIA bound efficiently (Fig. 3*A*), but TBP only marginally (Fig. 3*B*), to GST-PC4. Intriguingly, pol II was quantitatively retained on GST-PC4 beads but not on control glutathione *S*-transferase (GST) beads (Fig. 3*C*). On the other hand, no interaction of PC4 with TFIIB could be detected (Fig. 3*B*), in contrast to what was reported for a potential yeast homolog of PC4, SUB1/TSP1 (30). These results suggest that the T·B·F·II complex could be stabilized via direct pol II–PC4 interactions and, given the critical nature of the double-stranded DNA-binding region of PC4 for its coactivator function (9), that stabilization of the T·B complex could arise from indirect, DNA-mediated effects of PC4. Together with previous reports of PC4 interactions with various activators (7), it appears that PC4 stabilizes the multiprotein transcription complex via several pairwise interactions.

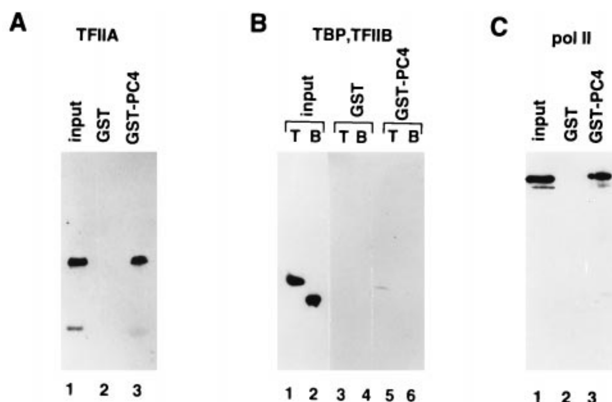

FIG. 3. DNA-independent interaction of PC4 with selected factors. Glutathione-Sepharose 4B beads containing GST-PC4 or GST alone were incubated with the indicated individual factors. After incubation for 30 min on ice and removal of unbound material, the beads were boiled in Laemmli sample buffer. After SDS/PAGE the polypeptides were transferred to nitrocellulose filters and blotted with appropriate antibodies. Twenty-five percent of the input is shown. (*A*) TFIIA. TFIIA was reconstituted with recombinant p12 and p55. The filter was probed with antibodies recognizing p55. (*B*) TBP (lanes 1, 3, and 5) and TFIIB (lanes 2, 4, and 6). The filter was probed with antisera recognizing recombinant TBP and TFIIB. (*C*) Pol II. The filter was probed with an anti-CTD mAb (8WG16).

PC4 Is Phosphorylated in the PIC. As $TAF_{II}250$, the largest subunit of TFIID (31), and the CDK activating kinase (*CAK*) component of TFIIH (32) each possess an intrinsic protein kinase activity, and because PC4 can be phosphorylated by a variety of kinases that include casein kinase II and protein kinase C (2, 33), we tested whether PC4 could be phosphorylated by TFIIH, TFIID, or $TAF_{II}250$. First, f:TFIID and a baculovirus-expressed epitope-tagged $TAF_{II}250$ that was purified by both affinity and conventional chromatographic steps, including gel filtration at high salt (34), were assayed for kinase activity with TFIIF and PC4 as substrates (Fig. 4*A*). In the range shown, neither TFIID nor $TAF_{II}250$, nor any of the substrate preparations, exhibited autophosphorylation (lanes

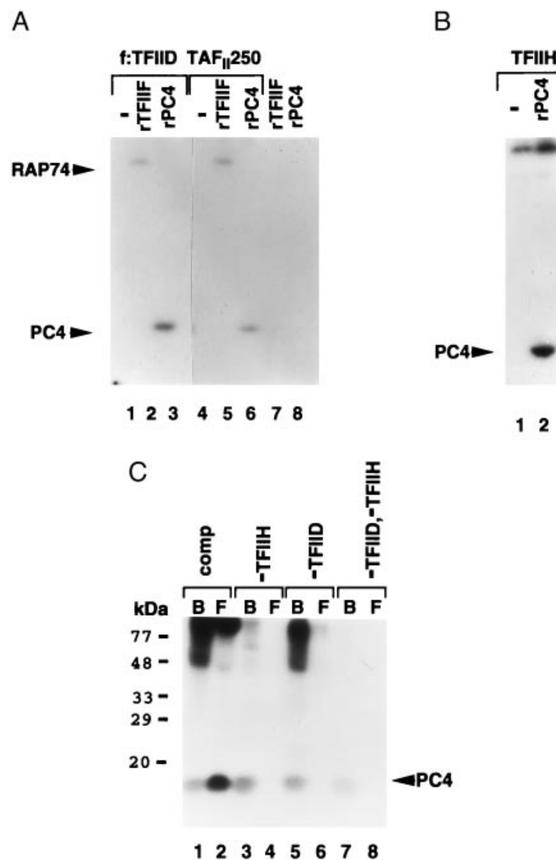

FIG. 4. Phosphorylation of PC4 by $TAF_{II}250$ and TFIIH in the PIC. (*A*) *In vitro* kinase reactions containing [γ-^{32}P]ATP were incubated with the indicated factor preparations (30°C for 30 min), precipitated with TCA, and analyzed by SDS/PAGE. Lanes 1–3, TFIID (50 ng); lanes 4–6, highly purified baculovirus-expressed recombinant $TAF_{II}250$ (10 ng). A total of 100 ng TFIIF (recombinant RAP74 and RAP30) was added to reactions in lanes 2, 5, and 7; 100 ng PC4 was added to lanes 3, 6, and 8. (*B*) Kinase reactions were done as in *A* with TFIIH (Ni-NTA-agarose fraction) alone (lane 1) or with 100 ng PC4 (lane 2). (*C*) Biotinylated dATP was incorporated into *Eco*RI-linearized pG5HML template by Klenow fill-in for immobilizing onto M280-streptavidin Dynabeads. Complete (comp) PICs were assembled in reactions in lanes 1 and 2 as in Fig. 1*C*, lane 16, except that the MonoS fraction of TFIIH was used. TFIIH (lanes 3 and 4) and TFIID (lanes 5 and 6) or both (lanes 7 and 8) were left out as indicated. After 45 min at 30°C, the complexes were washed four times in ice-cold transcription buffer. After incubation with [γ-^{32}P]ATP (30 min at 30°C) in transcription buffer, 100 μM ATP, CTP, and UTP were addd. Thirty minutes later the supernatant was removed, TCA precipitated, and the free (F) factors analyzed (lanes 2, 4, 6, and 8) by SDS/PAGE. The bound material on the beads (B) was boiled in Laemmli sample buffer and directly loaded on the gels (lanes 1, 3, 5, and 7). The PC4 band was identified by its size and on the basis of its appearance only upon addition of PC4 (data not shown). Molecular weight markers (kDa) are included.

1, 4, 7, and 8). As shown previously (31), both TFIID and $TAF_{II}250$ were able to phosphorylate the large subunit (RAP74), but not the small subunit (RAP30), of TFIIF (lanes 2 and 5). Most importantly, they also phosphorylated PC4 (lanes 3 and 6). Similarly, a highly purified preparation of TFIIH also efficiently phosphorylated PC4 (Fig. 4*B*). These data suggest that PC4 might undergo phosphorylation in the context of the PIC.

To evaluate this possibility we have used an immobilized template assay and asked if PC4 that is committed (bound) to these templates and subsequently released upon transcription becomes phosphorylated. For this purpose, biotinylated DNA templates containing GAL4 sites upstream of core promoter elements were immobilized on M280-streptavidin Dynabeads. Complete PICs were assembled in the presence of the activator (GAL-AH), PC4, pol II, and the general transcription factors including TFIID and TFIIH. The complexes were washed extensively to remove unbound factors and [γ-^{32}P]ATP was then added followed by other (unlabeled) nucleotides to initiate transcription. The supernatant and beads were finally analyzed for phosphorylated PC4 (Fig. 4*C*). The data revealed the presence of both residual template-bound PC4 (lanes 1) and free, phosphorylated PC4 (lane 2). Control experiments indicated that the appearance of phosphorylated PC4 in the supernatant was dependent on both TFIID (lanes 6 and 8) and TFIIH (lanes 4 and 8). These results show that PC4 can be phosphorylated in the PIC in a TFIID- and TFIIH-dependent manner and suggest further that PC4 release from the promoter may be coupled to this event (see *Discussion*).

DISCUSSION

Employing an activator-responsive transcription system reconstituted from essentially homogeneous preparations of general transcription factors, we have shown that, in contrast to basal transcription, PC4-mediated activated transcription is dependent both on TAFs in TFIID and on TFIIH. In their absence, PC4 strongly represses transcription while at the same time promoting the formation of minimal PICs. Furthermore, protein kinases in these multiprotein factors can phosphorylate PC4 in the context of the PIC.

Because the functional analysis (Fig. 1) indicating that a minimal complement of general factors is extremely susceptible to repression by PC4 is paradoxical to interaction studies (Figs. 2 and 3), indicating that PC4 actually facilitates minimal PIC formation, we infer that the PC4-stabilized complex represents an inactive PIC intermediate. It is also possible that the PC4 interactions that block minimal PIC function could be important for PIC assembly in the complete system. Therefore, TAF and TFIIH requirements could reflect a need to relieve the PC4 induced block and to allow either the formation or the function (initiation) of an active PIC.

Taken together with previous models of transcription activation mechanisms (6, 24), our results reveal a close interplay between the coactivator functions of TFIID and PC4, as well as that of TFIIH. Hence, coactivator-mediated transcription can be viewed, minimally, as a two-step process (Fig. 5). The PIC assembly events that follow activator-facilitated recruitment of TFIID to the core promoter, or TFIID recruitment itself, could be enhanced by PC4 through its ability to interact both with template (promoter) DNA and with multiple PIC components. In this capacity, PC4 might fulfill an architectural role analagous to those of the HMG-1/2 and HMG-1(Y) families of proteins (35, 36). However, the inherent stability of the resulting complex could preclude initiation of RNA synthesis. Therefore, in the second step, these contacts could be severed by a coupled mechanism that could, for example, involve phosphorylation of PC4, a modification previously shown to render PC4 incapable of interacting with DNA and activators (8, 9, 33).

Despite their role in phosphorylating PC4 in the PIC, the precise mechanism by which TAFs and TFIIH reverse PC4 inhibition is still an open question. The TAF requirement may also reflect their core promoter-specific effects (4, 37, 38), which could be exploited during PC4-directed PIC progression. Regardless of the precise role of TAFs, our results indicate that in their absence TBP-nucleated PICs that are otherwise active in transcription remain differentially sensitive to PC4 inhibition even in the presence of TFIIH. Given the

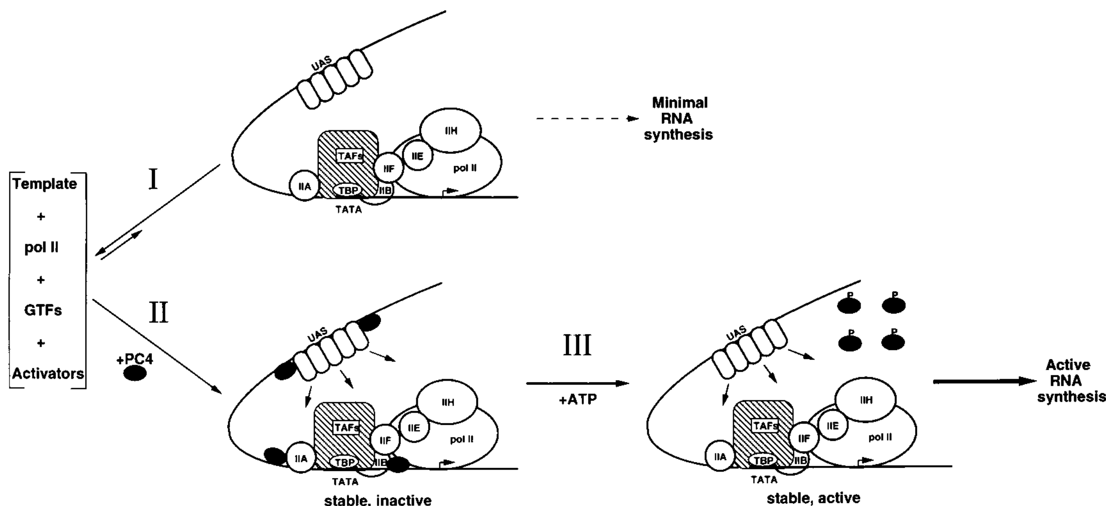

FIG. 5. Model for transcriptional activation involving interplay between TFIID, TFIIH, and PC4. Steps I and II outline the initial events in the assembly of the activated PIC. The complex in step I, formed in the absence of PC4, represents an unstable complex and leads to minimal transcription. Activators (shown here as an array of polypeptides) bound to the upstream activator sequence (UAS) mediate recruitment of the general transcription factors to the promoter. This process entails binding of TFIID (cross-hatched) to TATA elements followed by interactions of other factors either in a step-wise fashion or in the form of a pol II-associated complex. In step II, PC4 (solid) is proposed to stabilize the complex, possibly through its ability to engage in multiple interactions with the complex (see text). However, the resulting complex cannot initiate transcription unless a mechanism such as phosphorylation of PC4 by $TAF_{II}250$ or TFIIH dislodges PC4 and/or an ATP-hydrolysis dependent TFIIH helicase activity frees the pol II (step III). Only the first possibility (phosphorylation) is shown. In step II, a chaperone-like function of PC4 is imagined. Therefore, after its dissociation from the complex, the stabilizing influences of PC4 may be sustained through changes in DNA topology or rearrangements of the PIC polypeptides (not highlighted). Stoichiometry and spatial localization of PC4 is for illustrative purposes only.

relative magnitudes of the effects of TAFs and TFIIH, and in agreement with known functions of TFIIH in promoter clearance and elongation (32), it is likely that TFIIH plays a dominant role in this process. In addition to, or in lieu of, a role for TFIIH in PC4 phosphorylation, strand separation by the TFIIH helicase activity (32) could contribute to extrication of the nascent pol II complex from the PC4-anchored PIC intermediate. Given the strong correlation of PC4 function with its ability to bind DNA (9), this remains a likely possibility. Preliminary characterization of a PC4 derivative in which the major phosphorylation sites observed on natural intracellular PC4 pools are mutated (33) but whose coactivator activity is not diminished (data not shown) also points to a conditional requirement for PC4 phosphorylation.

In our model, activator-mediated recruitment of TFIIH (24) to a TAF-containing PIC in concert with PC4 stabilization of the complex would be manifested as overall activation of transcription. Implicit in this model is the idea that minimal transcription complexes (T·B·F·II) possess full transcription potential that is both suppressed by various regulatory constraints (e.g., specific TAFs, PC4) and unmasked by activators under appropriate conditions (e.g., the presence of TFIIH and specific TAFs). That TAFs and PC4 independently repress basal transcription in our purified system, but act synergistically to mediate activator function, underscores the complex mutual interdependence of these cofactors. Interestingly, the overall situation described in the model is reminiscent of that reported for activated transcription at prokaryotic σ^{54}-dependent promoters, where σ^{54}-holoenzyme is maintained in an inactive intermediate state until ATP hydrolysis by nitrogen regulatory protein C "unlocks" this conformation and allows progression into an active form (39). Because PC4 is representative of a diverse group of relatively abundant nuclear factors (upstream stimulatory activity) that participate in various DNA transactions and are likely to be routinely encountered by the transcription machinery (2), our multistep model may have more general implications for coactivator function.

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1. Roeder, R. G. (1996) *Trends Biochem. Sci.* **21,** 327–335.
2. Kaiser, K. & Meisterernst, M. (1996) *Trends Biochem. Sci.* **21,** 342–345.
3. Bjorkland, S. & Kim, Y.-J. (1996) *Trends Biochem. Sci.* **21,** 335–337.
4. Burley, S. & Roeder, R. G. (1996) *Annu. Rev. Biochem.* **65,** 769–799.
5. Verrijzer, C. P. & Tjian, R. (1996) *Trends Biochem. Sci.* **21,** 338–342.
6. Ptashne, M. & Gann, A. (1997) *Nature (London)* **386,** 569–577.
7. Ge, H. & Roeder, R. G. (1994) *Cell* **78,** 513–523.
8. Kretzschmar, M., Kaiser, K., Lottspeich, F. & Meisterernst, M. (1994) *Cell* **78,** 525–534.
9. Kaiser, K., Stelzer, G. & Meisterernst, M. (1995) *EMBO J.* **14,** 3520–3527.
10. Ge, H., Martinez, E., Chiang, C.-M. & Roeder, R. G. (1996) *Methods Enzymol.* **274,** 57–71.
11. Bitter, G. (1983) *Anal. Biochem.* **128,** 294–310.
12. Malik, S., Hisatake, K., Sumimoto, H., Horikoshi, M. & Roeder, R. G. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9553–9557.
13. Ohkuma, Y., Hashimoto, S., Wang, C. K., Horikoshi, M. & Roeder, R. G. (1995) *Mol. Cell. Biol.* **15,** 4856–4866.
14. Wang, Q., Kostrub, C. F., Finkelstein, A. & Burton, Z. F. (1993) *Protein Expression Purif.* **4,** 207–214.
15. DeJong, J., Bernstein, R. & Roeder, R. G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3313–3317.
16. Kretzschmar, M., Stelzer, G., Roeder, R. G. & Meisterernst, M. (1994) *Mol. Cell. Biol.* **14,** 3927–3937.
17. Malik, S. & Karathanasis, S. K. (1996) *Mol. Cell. Biol.* **16,** 1824–1831.
18. Maldonado, E., Ha, I., Cortes, P., Weis, L. & Reinberg, D. (1990) *Mol. Cell. Biol.* **10,** 6335–6437.
19. Goodrich, J. A. & Tjian R. (1994) *Cell* **77,** 145–156.
20. Jiang, Y., Yang, M. & Gralla, J. D. (1995) *J. Biol. Chem.* **270,** 27332–27338.
21. Holstege, F. C. P., van der Vliet, P. C. & Timmers, H. Th. M., (1996) *EMBO J.* **15,** 1666–1677.
22. Parvin, J. D. & Sharp, P. A. (1993) *Cell* **73,** 533–540.
23. Tyree, C. M., George, C. P., Lira-Devito, L. M., Wampler, S. L., Dahmus, M. E., Zawel, L. & Kadonaga, J. T. (1993) *Genes Dev.* **7,** 1254–1265.
24. Orphanides, G., Lagrange, T. & Reinberg, D. (1996) *Genes Dev.* **10,** 2657–2683.
25. Kokubo, T., Yamashita, S., Horikoshi, M., Roeder, R. G. & Nakatani, Y. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3520–3524.
26. Malik, S., Lee, D. K. & Roeder, R. G. (1993) *Mol. Cell. Biol.* **13,** 6253–6259.
27. Meisterernst, M. & Roeder, R. G. (1991) *Cell* **67**, 557–567.
28. Inostroza J. A., Mermelstein, F., Ha, I., Lane, W. S. & Reinberg, D. (1992) *Cell* **70** 477–489.
29. Auble D. T., Hansen, C. G. F., Mueller, Lane, W. S., Thorner, J. & Hahn, S. (1994) *Genes Dev.* **8,** 1920–1934.
30. Knaus, R., Pollock, R. & Guarente, L. (1996) *EMBO J.* **14,** 1933–1944.
31. Dikstein, R., Ruppert, S. & Tjian, R. (1996) *Cell* **84,** 781–790.
32. Svejstrup, J. Q., Vichi, P. & Egly, J.-M. (1996) *Trends Biochem. Sci.* **21,** 346–350.
33. Ge, H., Zhao, Y., Chait, B. & Roeder, R. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12691–12695.
34. Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M. & Roeder, R. G. (1993) *Nature (London)* **362,** 179–181.
35. Grosschedl, R., Giese, K. & Pagel, J. (1994) *Trends Genet.* **10,** 94–100.
36. Shykind, B. M. & Sharp, P. A. (1995) *Genes Dev.* **9,** 1354–1365.
37. Martinez, E., Chiang, C.-M., Ge, H. & Roeder, R. G. (1994) *EMBO J.* **13,** 3115–3126.
38. Shen, W.-C. & Green, M. R. (1997) *Cell* **90,** 615–624.
39. Geiduschek, E. P. (1997) *Science* **275,** 1614–1616.