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Trans-activation by the Human Aryl Hydrocarbon Receptor and Aryl Hydrocarbon Receptor Nuclear Translocator Proteins: Direct Interactions with Basal Transcription Factors

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SUMMARY

The aryl hydrocarbon (or dioxin) receptor (AhR) is a ligand-activated basic helix-loop-helix (bHLH) protein that heterodimerizes with the bHLH protein AhR nuclear translocator (ARNT) to form a complex that binds to xenobiotic regulatory elements in the enhancers of target genes. We used a series of fusion proteins, with a heterologous DNA-binding domain, to study independently the *trans*-activating function of the human AhR and ARNT proteins in yeast. The results confirm that both the human AhR and ARNT contain carboxyl-terminal *trans*-activation domains. The AhR has a complex *trans*-activation domain that is composed of multiple segments that function independently and exhibit varying levels of activation. Furthermore, these regions within the AhR cooperate when linked together, resulting in a synergistic activation of transcription. Fusion proteins of the AhR and ARNT *trans*-activation domains with the LexA DNA-binding domain, expressed in bacteria and purified to near-homogeneity, stimulated transcription of a minimal promoter *in vitro* in yeast nuclear extracts. Using this *in vitro* transcription assay, it was also possible to demonstrate that the AhR and ARNT *trans*-activation domains, in the absence of a DNA-binding domain, inhibited activated and basal transcription. Furthermore, *in vitro* the receptor bound selectively to the basal transcription factors, the TATA-binding protein and TFIIF, whereas ARNT bound preferentially to TFIIF. Taken together, these results suggest that AhR and ARNT activate target gene expression, at least in part, through direct interactions with basal transcription factors.

The AhR and its partner, ARNT, belong to a subfamily of helix-loop-helix proteins that include the *Drosophila* proteins SIM and PER as well as the recently cloned hypoxia-inducible factor-1α (Refs. 1–3 and references therein). The receptor is an enhancer binding protein that is activated by environmental pollutants and regulates the expression of a number of genes encoding phase I and phase II drug-metabolizing enzymes (4). The cytoplasmic AhR is found as a complex of several proteins, including hsp90, that maintain the receptor in an inactive state (5). The nuclear form of the receptor requires ligand-induced release of hsp90 and the other bound proteins and subsequent dimerization with ARNT (Refs. 6 and 7 and references therein). It is not clear whether ARNT is required for the receptor to translocate to the nucleus or the AhR binds ARNT once in the nucleus. However, DNA binding requires both AhR and ARNT. and this AhR/ARNT complex is localized to *cis*-acting xenobiotic response elements found upstream of AhR-regulated genes (4).

Models for activation of transcription by RNA polymerase II describe protein/protein interactions between DNA-bound activators and general factors assembled at the TATA box (8–10). Initiation of transcription by RNA polymerase II requires specific DNA binding by the TFIID complex and subsequent recruitment of the general or basal transcription factors TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH together with the polymerase to form the preinitiation complex (Refs. 9 and 10 and references therein). TFIID consists of the TBP and at least eight TATA-binding protein-associated factors (9, 10). Proteins that activate transcription (i.e., activators) may function by recruiting or modifying the activity of one or more of the general transcription factors, resulting in the formation of a more stable preinitiation complex. This function of activators is thought to be mediated by direct inter-

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ABBREVIATIONS: AhR, aryl hydrocarbon (or dioxin) receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; AX, aryl hydrocarbon receptor nuclear translocator *trans*-activation domain amino acids 685–744; bHLH, basic-helix-loop-helix; AhR:AB, aryl hydrocarbon receptor *trans*-activation domain amino acids 545–713; GR, glucocorticoid receptor; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; SRF, serum response factor; TBP, TATA-binding protein; BSA, bovine serum albumin; PBS, phosphate-buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

actions with one or more of the basal transcription factors and/or indirect contacts via coactivator or adapter proteins (for a recent review, see Ref. 10 and references therein). The specific mechanisms of AhR/ARNT activation of target genes are not clearly understood. Both the mouse AhR and human and mouse ARNT proteins possess carboxyl-terminal activation domains (11–14), and one possibility is that the carboxyl-terminal activation domains of the AhR and/or ARNT directly contact proteins in the transcriptional apparatus, thereby initiating transcription. Alternatively, the AhR/ARNT complex may aid in derepression of chromatin, which may be a requirement of activated transcription (Ref. 15 and references within).

A number of members of the steroid/thyroid hormone receptor superfamily, including the GR, estrogen, progesterone, vitamin D_3, and thyroid hormone receptors, have been shown to function as ligand-dependent regulators of appropriate reporter genes when expressed in yeast cells (Ref. 16 and references therein). Furthermore, we and others have reported that both the human and mouse AhRs activate transcription in yeast (6, 17, 18). The information obtained from these yeast models has extended our understanding of ligand-activated transcription factors as well as provided further evidence for the evolutionary conservation of transcription between higher and lower eukaryotes. In the current study, we show that both human AhR and ARNT have carboxyl-terminal *trans*-activation domains that function in yeast when fused to a heterologous DNA-binding domain. The AhR contains a complex *trans*-activation domain with multiple subdomains or modules that activate transcription independently and work together to cause a synergistic stimulation of transcription. Furthermore, with a cell-free transcription assay, the AhR and ARNT *trans*-activation domains, in the absence of a DNA-binding domain, were capable of squelching basal transcription. Binding studies identified TBP as a possible target for the receptor, whereas both the receptor and ARNT bound another of the basal transcription factors, TFIIF. These results strongly suggest that AhR and ARNT activate transcription, at least in part, via direct interactions with components of the general transcription machinery.

Materials and Methods

Yeast strain. Expression of recombinant proteins was carried out in the strain W303-1A (*MATa*, *ade2-1*, *can1-100*, *his3-11*, *15*, *leu2-3*, *112*, *trp1-1*, *ura3-1*). All transformations into this strain were done according to the lithium acetate transformation protocol as described previously (17).

General methods. PCRs were performed according to the manufacturer's instructions using the Expand Long Template PCR System (Boehringer-Mannheim Biochemica, Mannheim, Germany) that includes the thermostable *Taq* DNA polymerase and the proof-reading function of the *Pwo* DNA polymerase. Amplified products were purified from 1.5% agarose gels and subcloned according to standard molecular biological methods. The recombinant plasmids were confirmed by a combination of PCR analysis, automated DNA sequencing (Applied Biosystems, Norwalk, CT), and functional analysis of expressed proteins. These constructs generated proteins with the expected mobilities on SDS-PAGE and Western blot analysis (described below).

Oligonucleotides used for PCR reactions. The following oligonucleotides were used in the PCRs: D545N, 5′-GCGCGCGAGCTCTCTAGGCATTGATTTTGAAGACAT-3′; D600N, 5′-GCGCGCGAGCTCTCAACAGCAACAG TCCTTGGCTC-3′; D714N, 5′-GCGCGCGAGCTCTTGTACAGAGCTGGACTACCCTAT-3′; D600C, 5′-GCGCGCGAGCTCATATAATCTGAAGGTATGAAGGGA-3′; D713C, 5′-GCGCGCGAGCTCATTTTGGAATGTTGTGGTAATACA-3′; D848C, 5′-GCGCGCGAGCTCATCAGGAATCCACTGGATGTCAAATC-3′; A700N, 5′-GCGCGCGAGCTCTCGTGGATCTAACTTTGCTCCTG-3′; A786C, 5′-GCGCGCGAGCTCATGGGGGGAAACATAGTT-AGATCA-3′.

Construction of AhR and ARNT activation domain $LexA_{DBD}$ expression and reporter plasmids. For *in vivo* studies, all $LexA_{DBD}$ fusion plasmids were constructed in pRS315Lex. This galactose-inducible expression vector contains an insert corresponding to amino acids 1–87 of the bacterial LexA protein under the control of the promoter and terminator regions of a galactose-inducible 3-phosphoglycerate kinase gene.[2] PCR-amplified DNA fragments were ligated in frame into a single *Sac*I site 5′ to the $LexA_{DBD}$ sequence. For AhR and ARNT activation domains, the human AhR plasmid, phuAhR, and the human ARNT plasmid, pGEM7Z-ARNT, were used as templates in PCR reactions. Plasmids pAHR:ABC-, A-, B-, C, AB-, and BC-Lex were constructed by ligating the appropriate sequences of the human AhR amplified using the primer pairs D545N/D848C, D545N/D600C, D600N/D715C, D714N/D848C, D545N/D713C, and D600N/D848C, respectively. Plasmid pAX-Lex was constructed by ligating the human ARNT sequences corresponding to amino acids 685–774 amplified using the primers A700N and A786C. The GR-τ1 activation domain (amino acids 77–262) $LexA_{DBD}$ plasmid, pRS315τ1Lex, was a gift from Tova Almlöf (Karolinska Institute, Huddinge, Sweden).[3] The yeast reporter plasmid, pLGZ-Lex, contains a single LexA DNA-binding site (CTGTATGTACATA-CAG) cloned upstream of the *CYC1* promoter in plasmid pLGZ (19).

Construction of $Gal4_{DBD}$-AhR and -ARNT expression plasmids. The yeast strain SFY526 (17) was used to assay the activity of $GAL4_{DBD}$-AhR and -ARNT fusion proteins. The $GAL4_{DBD}$-AhR1-848 fusion protein was expressed from the plasmid pGDR, which has been described previously (17). The plasmid pGAHR115-545 encodes $GAL4_{DBD}$-AhR115-545 fusion protein and was constructed by digesting pGDR with BlnI and *Sal*I, filling in the ends with Klenow and religating. The resulting plasmid, pGAHR1-545, was digested with *Eco*RI and religated to yield the plasmid pGAHR115-545. $GAL4_{DBD}$-AhR545-848 fusion protein was expressed from pGAHR545-848 and was constructed by digesting pGDR with *Sma*I and *Sty*I, filling in the *Sty*I restriction site with Klenow and closing the plasmid with DNA ligase. To construct the $GAL4_{DBD}$-ARNT1-774 fusion protein expression plasmid, pGBAX, the *Bam*HI fragment encoding the full-length ARNT cDNA from the plasmid pGEM7Z-ARNT, was subcloned into the pGBT9 *Bam*HI site (17). $GAL4_{DBD}$-ARNT618-774 was expressed from the plasmid pGBAX618-774 and was constructed by subcloning a *Sty*I (filled in with Klenow) and *Sal*I fragment from pGBAX into the *Sma*I and *Sal*I sites of pGBT9.1. The plasmid pGBT9.1 was constructed by filling in the *Eco*RI site of pGBT9.

β-Galactosidase assays. Cells were grown overnight in minimal media containing 2% galactose and essential amino acids. On the next day, the cells were diluted into 50 ml of minimal media containing 2% galactose to an absorbance of 0.05 at 600 nm. The cells were incubated with shaking at 30° until the absorbance at 600 nm reached 0.25 (~6 hr). Then, 20 ml of the cell suspension was harvested for Western blot analysis (discussed below); the remaining cell suspension was harvested by centrifugation and resuspended in 150 μl of Z buffer (60 mM Na_2HPO_4, 40 mM Na_2HPO_4, 10 mM KCl, 1 mM $MgSO_4$, pH 7.0) containing 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride. Cells were ruptured through vortexing with glass beads for 20 min at 4°, and supernatants were transferred to new tubes. β-Galactosidase activity was measured by using the colorimetric substrate *o*-nitrophenyl galactoside as described previ-

[2] Details of this plasmid will be published elsewhere and are available on request.

[3] Details of the construction will be published elsewhere.

ously (17), and data are expressed as nmol of *o*-nitrophenyl galactoside converted/min/mg of protein. Protein concentrations were determined as described previously (17). The data are representative of the mean and standard deviation from triplicate samples of three separate experiments.

Construction of histidine-tagged AhR and ARNT activation domain bacterial expression plasmids. All histidine-tagged fusion plasmids were constructed in the pET19bm or pET-LexA$_{DBD}$ plasmids,[4] which were derived from the plasmid pET19b (Novagen, Madison, WI). Briefly, pET19bm has a modified polylinker to allow in-frame cloning into *Bam*HI and/or *Sac*I sites. pET-LexA$_{DBD}$ was constructed from pET19bm and contains cDNA corresponding to the bacterial LexA$_{DBD}$ (amino acids 1–87) cloned into the *Bam*HI site immediately 3′ of the *Sac*I site. The human AhR sequences corresponding to amino acids 545–713 amplified using the primers D545N and D713C were ligated into pET-LexA$_{DBD}$ and pET19bm to construct the plasmids pETAHR:AB-Lex and pETAHR:AB, respectively. The human ARNT sequences corresponding to amino acids 685–774 were amplified using the primers D700N and D786C and ligated into pET-LexA$_{DBD}$ and pET19bm to construct the plasmids pETAX-Lex and pETAX, respectively.

Production of recombinant receptor proteins. Histidine-tagged recombinant AhR and ARNT proteins were expressed in BL-21 bacteria by 1 mM IPTG induction and purified from the cleared cell lysate by ammonium sulfate precipitation and Ni^{2+} affinity chromatography. The eluted His-AhR:AB-Lex, His-AhR:AB, His-AX-Lex and His-AX were estimated to be ≥80% pure as determined from Coomassie blue staining of 15% SDS-polyacrylamide gels. Purified proteins were dialyzed against 25 mM HEPES, pH 7.6, 100 mM sodium acetate, and 1 mM dithiothreitol. His-AhR:AB* was extracted from bacterial inclusion bodies with 8 M urea in 10 mM Tris·HCl, pH 8.0/0.1 M NaH_2PO_4 and loaded directly onto the Ni^{2+}-NTA resin. The bound protein was then eluted in urea-containing buffer with decreasing pH, according to manufacturer's instructions (Novagen), and dialyzed in two steps against 4 M and then 0 M urea-containing dialysis buffer. All protein concentrations were determined according to the method of Bradford.

Cell free transcription assay. Squelching of basal transcription by AhR:AB and AX and activation of transcription by AhR:AB-Lex and AX-Lex in yeast nuclear extracts were as described previously (20). The reporter plasmid, pLexACG-, was constructed by cloning one adjacent LexA response element into the *Xho*I site of the plasmid pΔCG- (21).

Scintillation-microtitration plate-based protein/protein interaction assay. Purified recombinant *trans*-activation domains AhR:AB, AX, VP16$_{413-490}$, or SRF (GAL4-SRF$_{245-508}$) in binding buffer (20 mM HEPES, pH 7.6, 10% glycerol, 100 mM KCl, 0.2 mM EDTA, 5 mM β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride) were adsorbed to the surface of microtiter plate wells (ScintiStrips; Wallac Oy, Turku, Finland) for ≤72 hr at 4°. After removal of the activator solution, the wells were blocked for 12 hr at 4° with blocking buffer (binding buffer plus 5 mM $MgCl_2$ and 5 mg/ml BSA) and subsequently incubated with radiolabeled human general transcription factors for 90 min at room temperature, synthesized in rabbit reticulocyte lysates (Promega, Madison, WI), and diluted in binding buffer plus 5 mM $MgCl_2$ and 1 mg/ml BSA. The wells were subsequently washed four times (binding buffer plus 5 mM $MgCl_2$ and 1 mg/ml BSA), and the bound radioactivity counted directly in a micro-β counter (Wallac Oy). Bound proteins were then recovered in SDS sample buffer and analyzed by SDS-PAGE.

Western blots. Cell extracts were prepared by being boiled in SDS sample buffer for 10 min and removal of the insoluble debris through centrifugation at 14,000 rpm for 5 min. The proteins were resolved on a 15% polyacrylamide gel and transferred to nitrocellulose filters. Filters were blocked with 5% milk powder in PBS overnight at 4° and incubated with primary antibody (anti-LexA$_{DBD}$ rabbit polyclonal antibodies; a gift from Anthony Wright, Karolinska Institute, Huddinge, Sweden) diluted 1:2000 in PBS plus 0.05% Tween-80 (Sigma Chemical, Poole, Dorset, UK) ≤60 min at room temperature. After being washed (PBS plus 0.05% Tween-80), the filters were incubated with horseradish peroxidase conjugated anti-rabbit Ig (1:2000 dilution in PBS plus 0.05% Tween-80) and washed, and the proteins were visualized using the enhanced chemiluminescence system (ECL; Amersham International, Buckinghamshire, UK) and detected by autoradiography.

[4] pET19bm and pET-LexA$_{DBD}$ plasmids will be described in detail elsewhere.

Results

Identification of transcription activation domains in the human AhR and ARNT. Previous studies using cultured mammalian cells have revealed that the carboxyl-terminal halves of the mouse AhR and human ARNT contain regions critical for the transcriptional activation function of these proteins (11–14). Furthermore, we and others have reported that the mouse and human AhRs expressed in yeast (*Saccharomyces cerevisiae*) are capable of activating a reporter gene in response to a ligand (6, 17, 18). Removal of 303 amino acids from the AhR carboxyl terminus resulted in >90% loss of inducible activation, whereas this region alone is sufficient to confer transcriptional activation of a reporter gene in the context of Gal4$_{DBD}$ fusion proteins (Table 1). Thus, as observed in mammalian cells, the carboxyl-terminal region of the AhR harbors a strong *trans*-activation domain that functions in yeast. Table 1 also shows that *trans*-activation by ARNT is apparently restricted to the carboxyl-terminal 156 amino acids as this region is as active as the full-length protein in the *trans*-activation assay; in a separate study, deletion of these same amino acids from ARNT created a transcriptionally inactive protein when tested in a yeast two-hybrid assay (data not shown). Collectively, these results indicate that the carboxyl-terminal amino acids constitute the majority of the transcriptional activity of ARNT in yeast and are consistent with reports from other laboratories studying ARNT *trans*-activation in higher eukaryotes (11–13). To characterize in greater detail the domains responsible for the AhR and ARNT *trans*-activation in yeast, chimeric proteins were constructed by fusing specific carboxyl-terminal regions of human AhR or ARNT to the bacterial LexA DNA-binding domain (LexA$_{DBD}$) and tested for transcriptional activity from a LexA response element containing reporter gene *in vivo* (Fig. 1). This approach makes it possible

TABLE 1

Transactivation by GAL4$_{DBD}$-AhR and -ARNT fusion proteins: comparison of subdomain activities

GAL4$_{DBD}$ amino acids 1-147 were fused to either AhR amino acids 1-848, 115-545, or 545-848 or ARNT amino acids 1-774 or 618-774 and expressed in SFY526 yeast in the presence of 10 mM β-NF as described in Materials and Methods.

Fusion protein	Specific activity[a]	-Fold activation[a]
GAL4$_{DBD}$	0.15 ± 0.05	1
GAL4$_{DBD}$-AhR 1-848	9.6 ± 0.13	64.3 ± 0.87
GAL4$_{DBD}$-AhR 115-545	0.46 ± 0.002	3.1 ± 0.001
GAL4$_{DBD}$-AhR 545-848	1,976 ± 408	13,173 ± 2,720
GAL4$_{DBD}$-ARNT 1-774	131 ± 3	873 ± 20
GAL4$_{DBD}$-ARNT 618-774	181 ± 5	1,207 ± 33

[a] The specific β-galactosidase activity was calculated as described in Materials and Methods and used to determine the -fold activation relative to yeast expressing the GAL4$_{DBD}$ alone (mean ± standard deviation values from triplicate samples).

to identify regions within AhR and ARNT that are critical for *trans*-activation function and independent of known regions important for DNA binding and dimerization.

The carboxyl-terminal *trans*-activation domain of ARNT was further restricted to the last 89 amino acids, which stimulate a 12-fold increase in β-galactosidase activity when fused to the $LexA_{DBD}$ (Fig. 1C, *AX-Lex*). Because the AhR is not expressed in yeast and because the amino acids necessary for ARNT dimerization with the AhR have been deleted, this activity is independent of both the AhR and other potential ARNT dimerization partners. Taken together, these results indicate that ARNT contains a *trans*-activation domain located within the carboxyl-terminal 89 amino acids that functions in yeast.

The 303 carboxyl-terminal amino acids of human AhR stimulated a 182-fold increase in β-galactosidase activity when fused to the $LexA_{DBD}$ (Fig. 1C, *AhR:ABC-Lex*). Interestingly, the human AhR is composed of a strong *trans*-activation domain that can be separated into multiple weaker *trans*-activation domains. We divided the carboxyl terminus of the AhR into three regions based on primary amino acid sequence (Fig. 1A): AhR:A, amino acids 545–600, which is an acidic domain; AhR:B, amino acids 600–713, which is rich in glutamines; and AhR:C, amino acids 714–848, which contains high concentrations of proline, serine, and threonine. All three of these domains, AhR:A, AhR:B, and AhR:C, have moderate activities with 4-, 8-, and 23-fold activation, respectively, which are comparable to the activity of the ARNT activation domain (12-fold) but not as strong as that induced by the GR-τ1 activation domain (66-fold) (Fig. 1C). However, these three activation regions can act synergistically, with the activity induced by AhR:AB or AhR:BC as strong as that measured for the complete AhR carboxyl-terminal region (AhR:ABC-Lex), resulting in 190-, 255-, and 182-fold activation, respectively (Fig. 1C). Expression of the fusion proteins was measured qualitatively by Western blot analysis of yeast extracts using anti-$LexA_{DBD}$ antibodies (Fig. 2). Along with the expected bands (Fig. 2, *arrows*) for AhR-, ARNT-, and GR-τ1-$LexA_{DBD}$ fusion proteins, additional products were also detected. In the case of the AhR constructs and GR-τ1, this heterogeneity, which may in part result from degradation during extract preparation, is unlikely to significantly affect the above findings because the relative levels of full-length proteins are comparable (Fig, 2, *arrows*). However, although we cannot discount that the het-

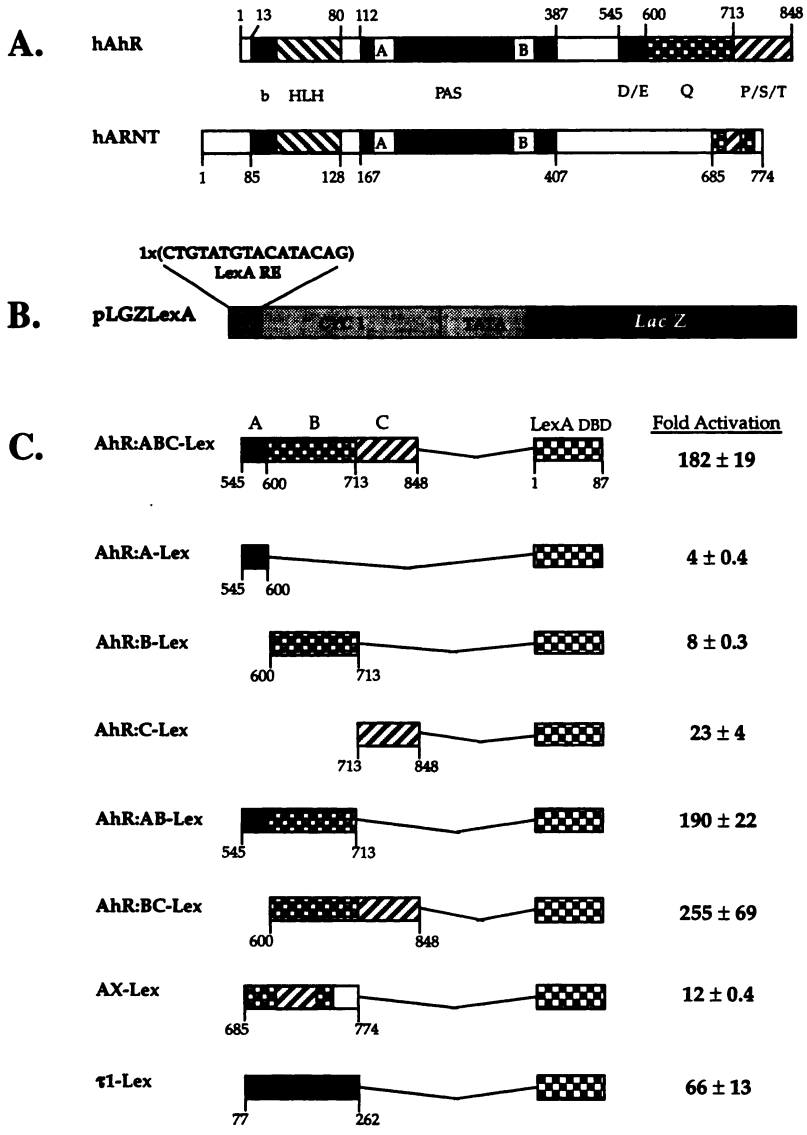

Fig. 1. Structural organization within the human AhR, ARNT, and yeast reporter gene and activity of chimeric constructs of the AhR-, ARNT-, and τ_1-$LexA_{DBD}$. A, Schematic of AhR and ARNT proteins (*hAhR* and *hARNT*) showing bHLH (*domains*); Per-ARNT-Sim (*PAS*) homology domain; and acidic (*D/E*), glutamine (*Q*), and proline-, serine-, and threonine- (*P/S/T*) rich regions. B, Schematic of the yeast DNA reporter construct used for the *in vivo* activation studies. C, Schematic AhR-, ARNT-, and τ_1-$LexA_{DBD}$ chimeras expressed in yeast and their -fold β-galactosidase activation relative to yeast expressing the $LexA_{DBD}$ alone (mean ± standard deviation values from triplicate samples of two independent experiments). $LexA_{DBD}$, amino acids 1–87; AhR:ABC, amino acids 545–848; AhR:A, amino acids 545–600; AhR:B, amino acids 600–713; AhR:C, amino acids 714–848; AhR:AB, amino acids 545–713; AhR:BC, amino acids 714–848; ARNT activation domain (AX), amino acids 685–774; and GR τ_1 activation domain, amino acids 77–262.

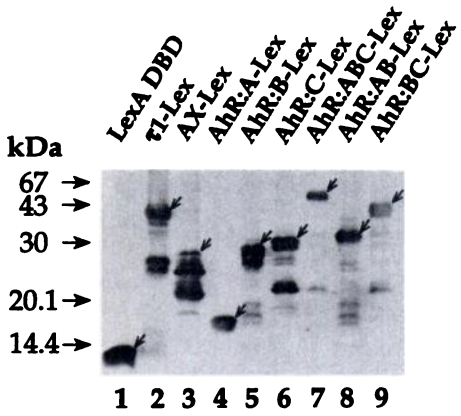

Fig. 2. Detection of fusion proteins by Western blotting. Whole cell yeast extracts were prepared from yeast transformed with the AhR-, AX-, and τ_1-LexA$_{DBD}$ chimeras. Fusion proteins were detected with polyclonal antiserum raised against the LexA$_{DBD}$. *Arrows*, positions of full-length proteins.

erogeneity of the ARNT-LexA$_{DBD}$ construct results in an underestimation of the relative activity of this protein, higher *trans*-activation activity by the AhR *trans*-activation domain relative to that of ARNT has also been reported in higher eukaryotes (11, 12, 14) and thus seems to be an inherent property of these proteins rather than an experimental artifact. These results indicate that the human AhR is composed of several activation regions that, when combined, constitute two strong and overlapping activation domains: one spanning amino acids 545–713 (AhR:AB) and the other spanning amino acids 600–848 (AhR:BC).

Carboxyl-terminal amino acids of the AhR and ARNT activate transcription *in vitro*. To begin to study the mechanisms involved in AhR:AB- and AX-dependent transcription activation, we used a yeast nuclear extract *in vitro* transcription assay. Transcription was measured using reporter genes consisting of a G-free cassette downstream of sequences from the *CYC1* promoter with an upstream LexA DNA response element (Fig. 3B). For these studies, the ARNT (AX) and AhR:AB *trans*-activation domains were expressed in *Escherichia coli* as histidine-tagged fusion proteins with the LexA$_{DBD}$ (Fig. 3A). The proteins were purified to >80% homogeneity as judged by Coomassie blue staining of polyacrylamide gels (Fig. 3C). Fig. 4A shows that the addition of increasing amounts of recombinant AX-Lex strongly stimulated transcription from the 1xLexA-*CYC1* promoter. Similarly, the AhR:AB-Lex protein induces a dose-dependent transcriptional response when added to the yeast nuclear extract (Fig. 4B). The levels of activation observed for these proteins were comparable to the activity seen with LexA fusions of the GR-τ1 and the herpes simplex viral protein VP16 *trans*-activation domains.[5] These results indicate that the yeast nuclear extract contains proteins necessary for the activation of transcription by AhR:AB-Lex and AX-Lex and that this system can be used to dissect the mechanisms of *trans*-activation by AhR and ARNT.

The AhR and ARNT activation domains contact limiting factors in the transcription machinery. To address the question of whether limiting target factors are required for AhR and ARNT *trans*-activation, we performed squelching experiments. Previous studies have reported that high levels of transcriptional activators can inhibit, or squelch, transcription by sequestering limiting cellular cofactors or general transcription factors (Refs. 16, 20, and 22 and references within). For these studies, AX and AhR:AB without the LexA$_{DBD}$ were expressed in *E. coli* and purified to >80% homogeneity as judged by Coomassie blue staining of polyacrylamide gels (Fig. 3, A and C). Fig. 5A shows that the addition of increasing amounts of AX to the nuclear extracts inhibits transcriptional activation by AX-Lex. Similarly, the addition of increasing amounts of AhR:AB caused squelching of AhR:AB-Lex-induced transcription (Fig. 5B). The squelching or inhibition by AhR was not observed when AhR:AB was purified by urea extraction of the insoluble *E. coli* proteins,

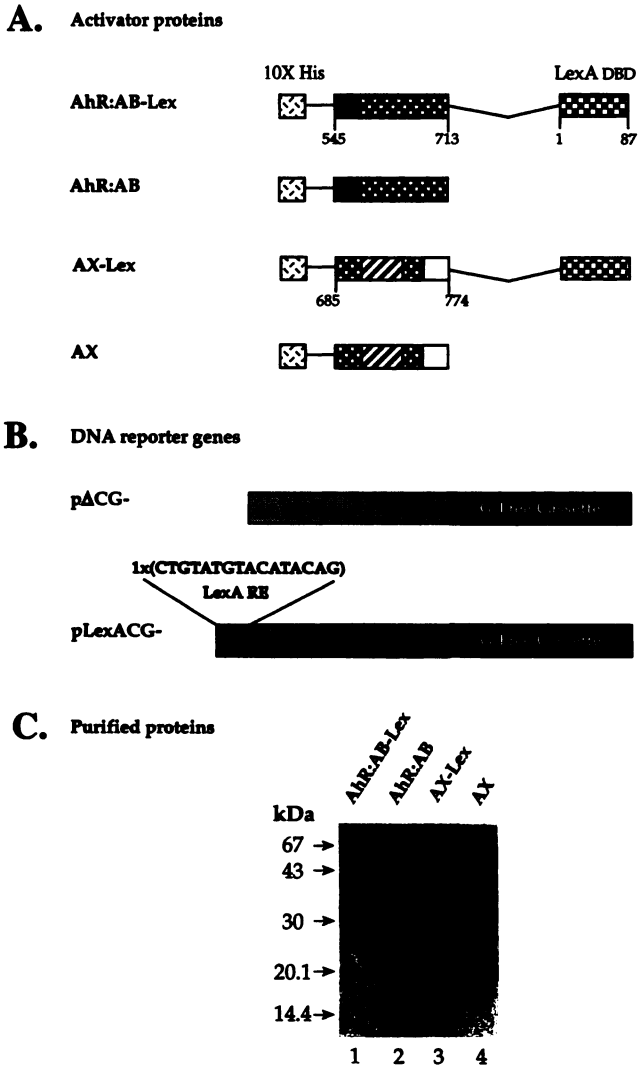

Fig. 3. Schematic of the activator proteins AhR:AB-Lex and AX-Lex, the activation domains AhR:AB and AX, the G-free cassette reporter construct, pLexACG-, and purified recombinant proteins. A, Histidine-tagged (*10X His*) activators: LexA$_{DBD}$, amino acids 1–87; AhR:AB, amino acids 545–713; and ARNT activation domain (AX), amino acids 685–774. B, Schematic of the yeast DNA reporter construct used for the *in vitro* activation studies. C, Coomassie blue-stained SDS-polyacrylamide gel of bacterially expressed purified recombinant proteins. The activator proteins AhR:AB-Lex and AX-Lex (*lanes 1 and 3*) and the activation domains AhR:AB and AX (*lanes 2 and 4*) were expressed in *E. coli* and purified on Ni^{2+} columns for use in the *in vitro* transcription assays. The proteins seen here were judged to be >80% pure.

[5] I. J. McEwan, unpublished observations.

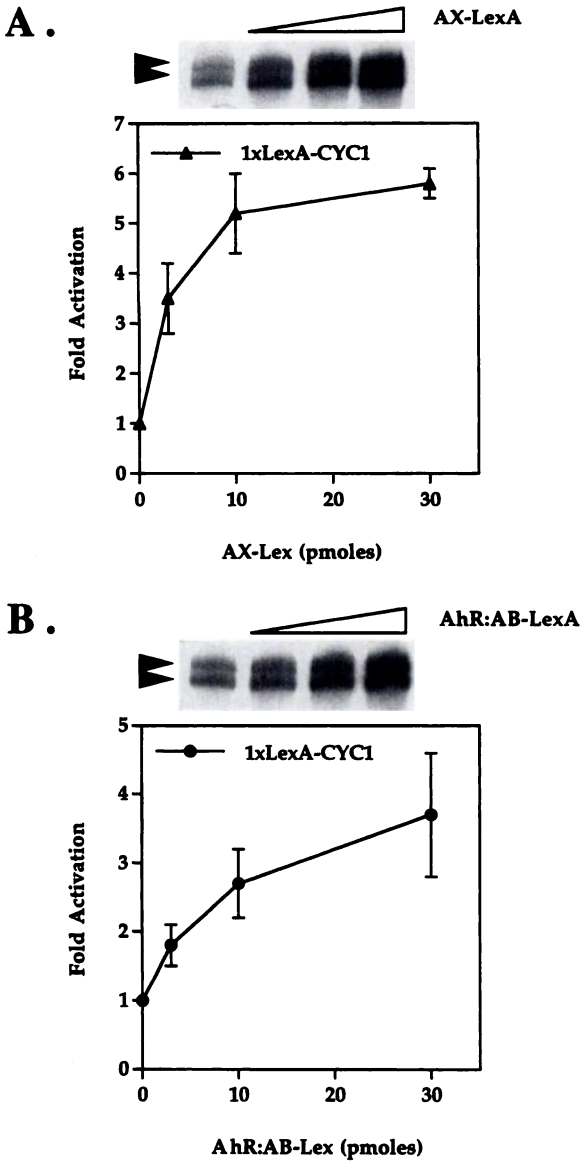

Fig. 4. AhR:AB-Lex and AX-Lex activate transcription *in vitro*. Yeast nuclear extract, 100 ng of pLexACG- template DNA, nucleotide triphosphates, and 0–30 pmol of AhR:AB-Lex or AX-Lex were incubated for 55–60 min. Specific RNase T_1-resistant transcripts were resolved on a 7% polyacrylamide/7 M urea denaturing gel, visualized by autoradiography, and quantified by using a Bio-imaging analyzer BAS2000 (Fuji Film). Representative examples of the transcription levels observed are shown above the respective graphs and were printed using a BAS HG-printer (Fuji Film) after adjustment of the background threshold. Arrowheads, the two specific RNase T_1-resistant transcripts resulting from initiation at the *CYC1* promoter (see Ref. 21). The data were calculated as the mean ± standard deviation for three experiments and plotted as the -fold activation relative to basal activity.

indicating that this squelching is not the result of nonspecific interference from the recombinant protein but instead requires the addition of a functional activation domain (see below and data not shown). Furthermore, the concentration of protein required for squelching is comparable to that needed for activation from a single LexA-binding site by the LexA fusion proteins (compare Figs. 4 and 5). Thus, the interactions occurring during squelching are likely to be functionally relevant for AhR- and ARNT-dependent transcription initiation. Similar findings have been reported for the viral activator protein VP16 and the GR-τ1 *trans*-activation domains (Ref. 20 and references therein). These experiments indicate that the isolated AhR:AB or ARNT (AX) *trans*-activation domains are capable of binding and competing for target factors needed for activation by the DNA-bound AhR:AB-Lex or AX-Lex, respectively.

If AhR and ARNT activate transcription through a similar mechanism, then they might be expected to have one or more target factor in common. To address this possibility, we investigated whether ARNT would squelch AhR-LexA-activated transcription, and *vice versa*. The results presented in Fig. 5C show that *in vitro* activation by AhR-LexA is inhibited by the addition of increasing amounts of the isolated ARNT activation domain to the assay. Furthermore, activation by AX-Lex is inhibited, albeit less effectively, by the addition of increasing amounts of AhR:AB activation domain to the assay (Fig. 5D). This reciprocal inhibition of *trans*-activation by AhR:AB on AX-Lex activation and AX inhibition of AhR:AB-Lex activation supports a model in which both AhR:AB and AX transcriptional activity is mediated through one or more common target factor(s). This approach has been used by others as evidence for the existence of common coactivators (23, 24). However, an alternative explanation for the above findings would be that the factor or factors being sequestered by AhR:AB and ARNT are components of the general transcriptional machinery.

The AhR and ARNT activation domains contact components of the general transcription machinery. To investigate the mechanism of AhR and ARNT activation further, we used the *in vitro* squelching assay to determine whether the AhR:AB or ARNT (AX) *trans*-activation domains are making direct contacts with the general transcriptional machinery. We performed the squelching assay in which transcription was measured from a reporter gene consisting of a G-free cassette downstream of sequences from the TATA elements from the *CYC1* promoter without any upstream LexA DNA response elements (i.e., a basal promoter) (Fig. 3B). Any transcription from this basal promoter is due to the general transcription apparatus alone. Fig. 6 shows that the addition of AhR:AB or AX caused a significant decrease in basal transcription from this reporter gene. Furthermore, the addition of AhR:AB isolated from bacterial inclusion bodies by urea extraction and subsequent dialysis showed a much reduced ability to squelch basal transcription (30% compared with 70% for nondenatured protein; Fig. 6C). This is consistent with the integrity of the *trans*-activation domain being critical for the squelching activity. These results strongly suggest that the AhR:AB and AX *trans*-activation domains directly contact one or more of the factors required for basal transcription, thus accounting for, at least in part, the inhibition observed in Fig. 5.

Direct binding of the AhR:AB *trans*-activation domain with the basal transcription factors TBP and TFIIF. As a first step in identifying which of the general transcription factors binds to the AhR:AB *trans*-activation domain, *in vitro* binding studies were carried out with immobilized activator proteins and radiolabeled human basal transcription factors. Bacterially produced AhR:AB or BSA (Sigma Chemical) control were allowed to attach to the surface of a microtiter well and were incubated with radiolabeled human basal transcription factors TFIIB, TBP, TFIIEα, TFIIEβ, TFIIF (RAP30), and TFIIF (RAP74). After

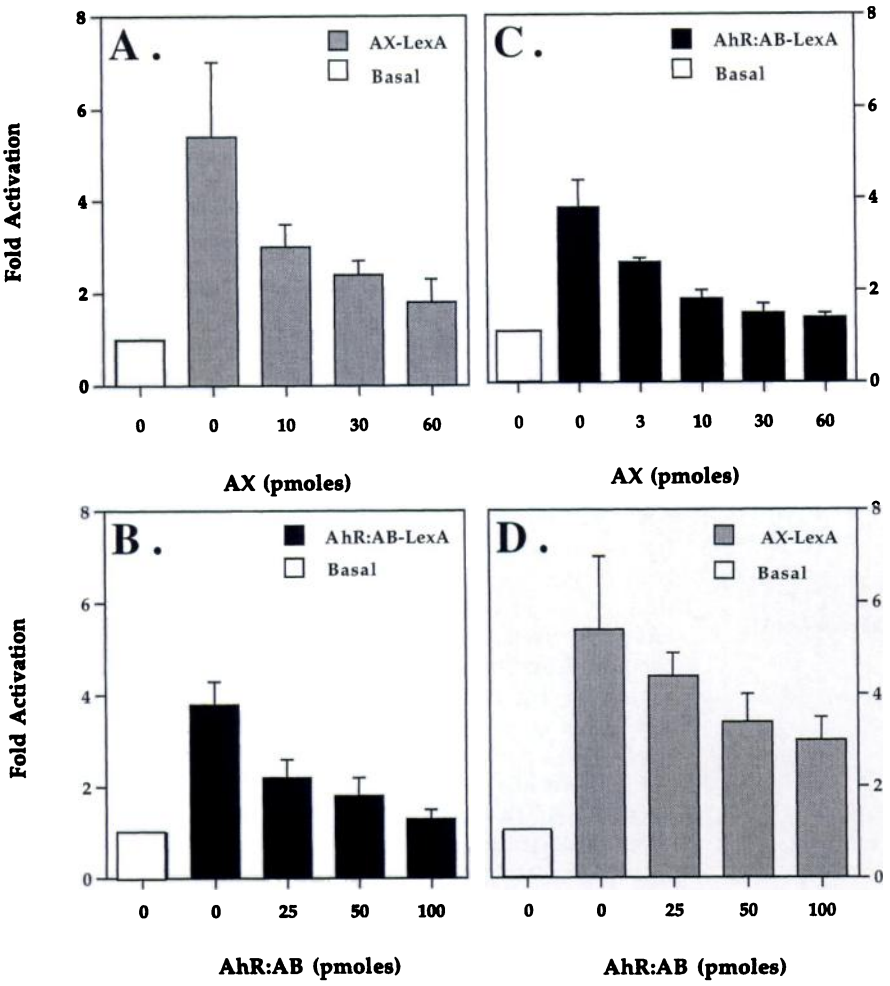

Fig. 5. Squelching of activated transcription by AhR:AB and AX. Transcriptional activation by the addition of 20 pmol of AX-Lex (A and D) or 25 pmol of AhR:AB-Lex (B and C) to yeast nuclear extract plus 100 ng of pLexACG- template DNA. Both AX-Lex- and AhR:AB-Lex-activated transcriptions were inhibited by the addition of increasing amounts of purified AX to the reactions (A and C, respectively). Transcripts were analyzed and quantified as described in the legend to Fig. 4, and the mean ± standard deviation values from up to five experiments were plotted relative to basal levels of transcription (set at 1). Both AhR:AB-Lex- and AX-Lex-activated transcription were inhibited by the addition of increasing amounts of purified AhR:AB to the reactions (B and D, respectively). The mean ± standard deviation values from up to five experiments were plotted relative to basal levels of transcription (set at 1).

incubation, the wells were washed, bound radioactivity was measured directly (Fig. 7A), and bound protein was recovered from the wells in SDS sample buffer and analyzed by SDS-PAGE (Fig. 7B). Under these conditions, a selective interaction was observed between AhR:AB domain and TBP and the RAP 30 and 74 subunits of TFIIF (Fig. 7A). In contrast, little or no significant binding was seen with TFIIB, TFIIEα, or TFIIEβ (Fig. 7A). In general, analysis of the bound radiolabeled material by SDS-PAGE qualitatively reflects the relative amounts of radioactivity measured directly (compare Fig. 7, A and B). It is of particular note that although translation of RAP74 *in vitro* results in multiple labeled products, it seems that the full-length protein is preferentially bound by AhR:AB (Fig. 7B). Although binding to the ARNT *trans*-activation domain was generally elevated with all the basal factors tested (relative to BSA), there was some selectivity observed for the subunits of TFIIF (Fig. 7C) and, to a lesser extent, TFIIB (relative binding = 2.8 ± 0.5).

To evaluate the significance of the above interactions, we compared the binding of AhR:AB and ARNT (AX) with previously reported interactions between VP16 and TBP (Refs. 9 and 25 and references therein) and SRF and TFIIF (30). The binding of AhR:AB to TBP was comparable to that seen for the strong viral activator $VP16_{413-490}$ *trans*-activation domain (Fig. 7C). Similarly, the binding of both AhR:AB and ARNT to the RAP74 subunit of TFIIF was as good, if not better, than that for the SRF carboxyl-terminal *trans*-activation domain (amino acids 245–508). Furthermore, under identical conditions, little or no significant binding of the other basal transcription factors with the $Gal4_{DBD}$-SRF or $Gal4_{DBD}$ proteins was observed (Fig. 7C and data not shown). Taken together, the results of the binding and functional studies strongly suggest that the *trans*-activation domains of the AhR and ARNT interact with components of the general transcriptional machinery and identify TBP and/or TFIIF as possible targets, respectively.

Discussion

We used *in vivo* and *in vitro* yeast transcription systems to characterize the *trans*-activation domains of the human AhR and ARNT proteins. By fusing carboxyl-terminal portions of these proteins onto a heterologous DNA-binding domain, we were able to characterize their activities independent of their DNA-binding and dimerization domains. The results show that the activation domain of the human AhR and ARNT reside in their carboxyl termini and are consistent with several recent reports that have described carboxyl-terminal *trans*-activation domains in both the mouse AhR and the human and mouse ARNT (11–14). Furthermore, *in vitro* basal and activated transcription studies revealed that the AhR and ARNT *trans*-activation domains compete for limiting factors required for basal transcription. Binding studies revealed that the AhR:AB *trans*-activation domain selec-

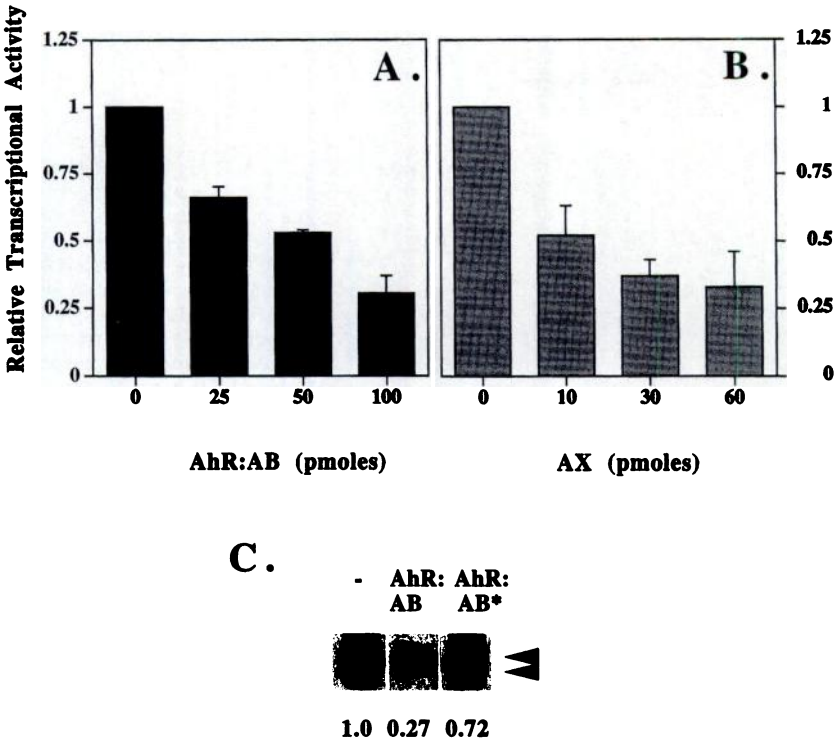

Fig. 6. Squelching of basal transcription by AhR:AB and AX. Basal transcription was measured in yeast nuclear extract containing 100 ng pΔCG- or pLex-ACG- template DNA. Transcripts were analyzed and quantified as described in the legend to Fig. 4. The addition of increasing amounts of AhR:AB (A) or AX (B) to the reactions inhibited basal transcription. The mean ± standard deviation values for at least three experiments were plotted relative to basal levels of transcription from reactions without AhR:AB or AX added (set at 1). C, Representative data showing that addition of 100 pmol of AhR:AB purified from bacteria by urea extraction (AhR:AB*) resulted in significantly less squelching of basal transcription compared with AhR:AB purified under nondenaturing conditions, with the relative levels of transcription (shown below each lane) equivalent to 0.30 ± 0.1 (eight observations) and 0.69 ± 0.06 (three observations), respectively.

tively bound TBP and RAP30 and RAP74 subunits of TFIIF, which is consistent with a model for AhR-dependent *trans*-activation involving direct binding to basal transcription factors (Fig. 8). In this model, the role of ligand and/or binding of ARNT will result in the unmasking of the carboxyl-terminal *trans*-activation domain of the receptor, which will then act to recruit components of the general transcription machinery to the promoter and increase target gene transcription. The model also illustrates the probability that other factors, including coactivators, are also involved (discussed below) and that once bound to the DNA the *trans*-activation domain of ARNT can also participate in the recruitment of the general transcription machinery.

TBP plays a central role in the assembly of the preinitiation complex and has been shown to be bind to a number of viral and cellular transcriptional activators, including members of the steroid/thyroid nuclear receptor superfamily (Ref. 25 and references therein; 26, 27). The interaction of the AhR with TBP suggests that the receptor functions at an early stage in the initiation of transcription and involves possible recruitment of TBP to the TATA element and/or stabilization of the TBP/TATA complex.

The interaction of both AhR and ARNT *trans*-activation domains with the subunits of TFIIF is of particular note. TFIIF interacts with both the RNA polymerase II and another basal factor, TFIIB. The role of TFIIF in initiation seems to involve recruitment of the polymerase specifically to the promoter sequences and thus prevents nonspecific DNA binding by the polymerase enzyme (Ref. 9 and references therein). In addition, TFIIF has been reported to be associated with RNA polymerase II in the elongation complex (28). It is therefore tempting to speculate that in addition to functioning during transcriptional initiation, the AhR and ARNT proteins may play a role during elongation of the nascent RNA. Only one other cellular activator, SRF, has been reported to bind to this basal transcription factor. The carboxyl-terminal *trans*-activation domain of SRF specifically bound to the RAP74 subunit of TFIIF, and TFIIF was shown to be important for transcriptional activation by SRF (29, 30). There is no striking homology between AhR or ARNT and SRF at the level of amino acid sequence, although there is some limited homology between part of the Q-rich domain of AhR (amino acids 593–639) and amino acids 427–474 of SRF. However, this region of SRF seems to lie outside of the RAP74-binding domain in that deletion of amino acids 412–432 of the SRF *trans*-activation domain (amino acids 412–508) severely impaired RAP74 binding (30). Clearly, more experiments will be needed to map the precise requirements for AhR and ARNT binding to TFIIF.

The binding of AhR and ARNT to known components of the general transcriptional machinery is compatible with the ability of these proteins to squelch both basal and activated transcription. However, attempts to overcome the squelching of basal transcription by AhR:AB and AX, with bacterially produced TBP, or TFIIF have been unsuccessful.[6] The latter findings suggest that both factors combined or additional factors may be important for AhR:AB- and ARNT-dependent *trans*-activation and squelching. These additional factors could be coactivators or additional basal transcription factors (Fig. 8). Interestingly, AhR:AB was less efficient at competing for factors required for AX-Lex activity than for either its own (AhR:AB-Lex) or the basal activity (no activator). This may reflect a lower affinity of AhR:AB compared with ARNT for a common target, or it may indicate that although AhR and ARNT have one or more factors in common, ARNT can also function by a distinct pathway. Further research will be required to distinguish between these possibilities. Significantly, both TBP and TFIIF exist in cells, from yeast to

[6] I. J. McEwan, unpublished observations.

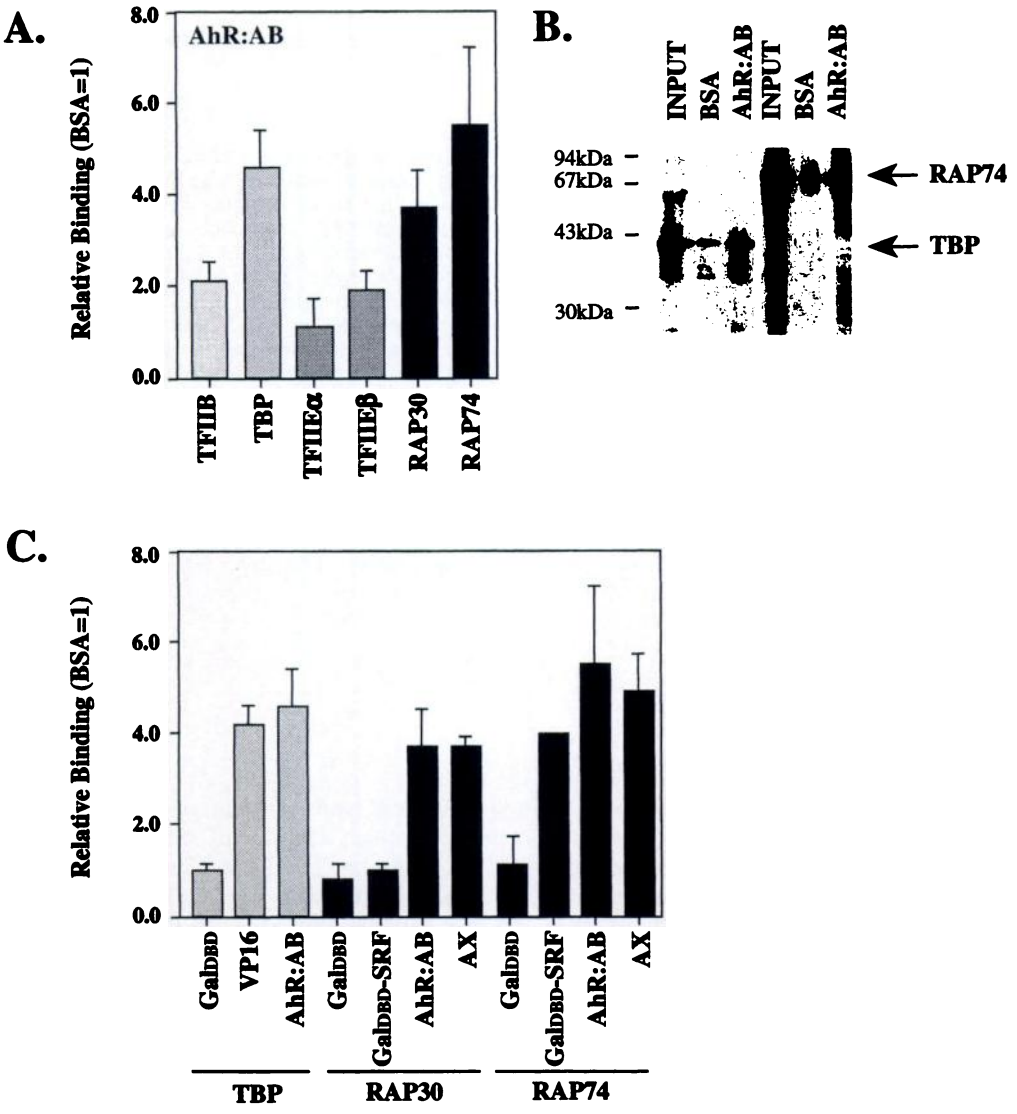

Fig. 7. AhR:AB and ARNT *trans*-activation domains bind components of the general transcription machinery. A, Binding of radiolabeled human general transcription factors (5 μl of reticulocyte lysate) to the AhR (AhR:AB) *trans*-activation domain. The mean ± standard deviation values (five to seven experiments) are plotted relative to the BSA control set to 1. B, SDS-gel of radiolabeled TBP, and RAP 74 bound to AhR:AB *trans*-activation domain. Input is equivalent to 3–4% of the starting material (150 μl of buffer plus 5 μl of translated protein). C, Comparison of the binding of AhR:AB, the carboxyl-terminal *trans*-activation domain of the viral activator VP16 and the Gal4 DNA-binding domain (*DBD*) with radiolabeled TBP, and the binding of AhR:AB, ARNT, and GAL-SRF *trans*-activation domains and Gal4DBD with radiolabeled TFIIF (RAP30 and RAP74) (as described in A). VP16, amino acids 413–490; GAL_{DBD}, amino acids 3–92; and GAL-SRF, GAL_{DBD} plus SRF, amino acids 245–508. The mean ± standard deviation (four to six experiments) are plotted relative to the BSA control set to 1.

humans, as part of the multiprotein complexes TFIID and holo-RNA polymerase, respectively (31–35). Although these complexes seem to be biochemically distinct, they share the common property of being necessary for activated levels of transcription (35–40). It will therefore be of importance to determine which, if any, other components of these complexes play a role in the AhR and ARNT *trans*-activation functions.

We found that the AhR is composed of two independent *trans*-activation domains with similar activities. These two *trans*-activation domains are strong activators and are composed of regions that are only moderately active but activate transcription synergistically when combined. The synergy measured by combining AhR:A with AhR:B (AhR:AB) or AhR:B with AhR:C (AhR:BC) might result from the stabilization of a secondary structure that cannot be assumed by any of these regions alone. Alternatively, synergism might arise through multiple contacts between the AhR *trans*-activation domains and the general transcriptional apparatus or potential coactivator(s) (22). We are examining both of these possibilities.

While the current work was in progress, similar findings were reported for the mouse AhR *trans*-activation domain (14). A striking difference was the activity of the region in the mouse receptor equivalent to AhR:A. This acidic region in the mouse AhR resulted in an ~100-fold stimulation of transcription in cultured mammalian cells (14) compared with a modest 4-fold activity seen with the human AhR:A in yeast cells (Fig. 1C). The reasons for this difference might be structural differences between the mouse and human receptors; e.g., a larger fragment of the mouse receptor (amino acids 490–593) was used (equivalent to human AhR residues 500–600). Alternatively, the potency of this domain may be enhanced through contacts with a mammalian specific coactivator that is not expressed in yeast. Synergism by subdomains was also observed for the mouse AhR expressed in mammalian cells, particularly for the regions equivalent to AhR:BC (Ref. 14 and Fig. 1C). However, synergism for the mouse A and B regions was much less pronounced, probably as a result of the relatively high activity of the mouse AhR:A subdomain alone.

Activated transcription is a multistep process involving initial binding to DNA by the activator with subsequent interaction with the general transcription factors, coactivators, or both. We used a yeast *in vitro* transcription assay to begin to identify which components of the transcriptional machinery are targeted by the AhR and ARNT. The activa-

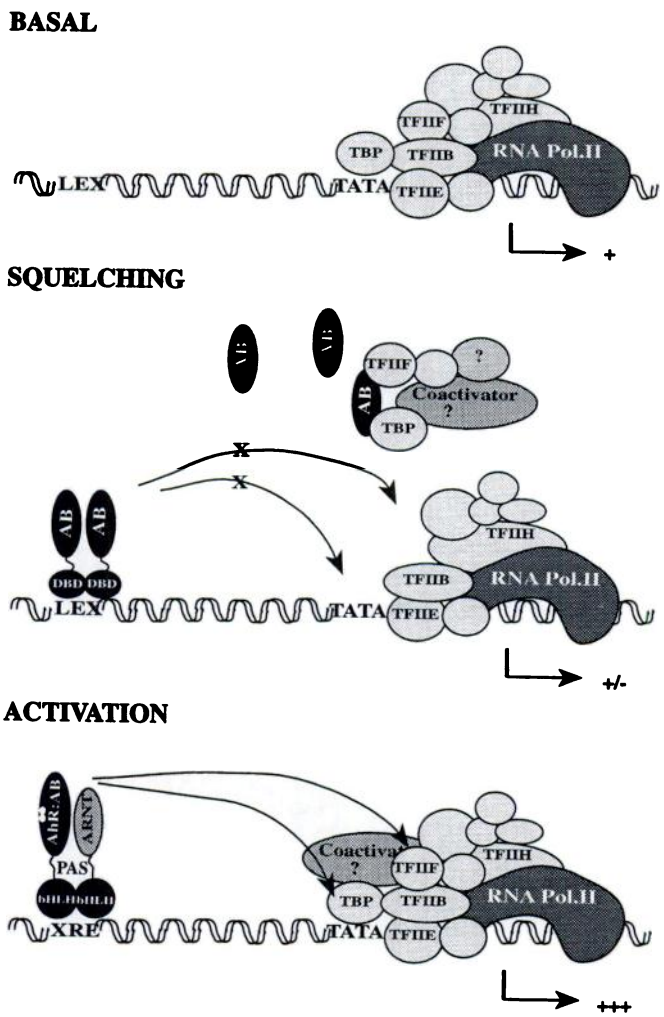

Fig. 8. Model for the mechanism of *trans*-activation by AhR and ARNT. *Top*, The polymerase and basal transcription factors are sufficient for the initiation of basal levels of transcription *in vitro*. *Middle*, The AhR *trans*-activation domain in solution can compete for limiting factors (squelching) required for activation and basal transcription. The results presented are consistent with the *trans*-activation domain sequestering a complex of factors that includes TBP, TFIIF, and as-yet-unidentified factors. For clarity, only the AhR *trans*-activation domain is shown, but the model is also applicable to the mechanism of action of ARNT involving binding to TFIIF and as-yet-unidentified factors. *Bottom*, Transcription activation by the DNA-bound AhR/ARNT complex is proposed to involve direct contacts with the basal transcriptional machinery via binding to TBP and TFIIF. *Light circles next to the AhR*, bound ligand. In addition, interactions with coactivators or adapters probably are also critical for this function. See text for full discussion.

tion domains of AhR and ARNT, when fused to a heterologous DNA-binding domain, were able to activate transcription of a reporter gene *in vitro*. The isolated *trans*-activation domains AhR:AB and ARNT were able to compete for one or more factors required for activation by DNA-bound AhR:AB and ARNT. Furthermore, both AhR:AB and AX squelch basal transcription; therefore, at least part of the mechanism of AhR and ARNT activation involves interactions with one or more of the general transcription factors. Consistent with this, the *trans*-activation domains of AhR and ARNT were found to bind to the basal transcription factors TBP and/or TFIIF. The ability to reconstitute the *trans*-activation functions of the receptor and ARNT *in vivo* and *in vitro* in yeast provides an important tool with which to investigate the mechanisms of AhR- and ARNT-dependent *trans*-activation.

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References

1. Burbach, K. M., A. Poland, and C. A. Bradfield. Cloning of the Ah receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc. Natl. Acad. Sci. USA* **89**:8185-8189 (1992).
2. Reyes, H., S. Reiz-Porszasz, and O. Hankinson. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science (Washington D. C.)* **256**:1193-1195 (1992).
3. Wang, G. L., B. H. Jiang, E. A. Rue, and G. L. Semenza. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-pas heterodimer regulated by cellular O2 tension. *Proc. Natl. Acad. Sci. USA* **92**:5510-5514 (1995).
4. Whitlock, J. P., Jr. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* **30**:251-277 (1990).
5. Perdew, G. H., and A. Poland. Purification of the Ah receptor from C57BL/6J mouse liver. *J. Biol. Chem.* **263**:9848-9852 (1988).
6. Carver, L. A., V. Jackiw, and C. A. Bradfield. The 90-kda heat shock protein is essential for Ah receptor signaling in a yeast expression system. *J. Biol. Chem.* **269**:30109-30112 (1994).
7. McGuire, J., M. L. Whitelaw, I. Pongratz, J.-Å. Gustafsson, and L. Poellinger. A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-helix dioxin receptor. *Mol. Cell. Biol.* **14**:2438-2446 (1994).
8. Roeder, R. G. The complexities of eukaryotic transcription initiation: regulation of preinitiation complex assembly. *Trends Biochem. Sci.* **16**:402-408 (1991).
9. Conaway, R. C., and J. W. Conaway. General initiation factors for RNA polymerase II. *Annu. Rev. Biochem.* **62**:161-190 (1993).
10. Zawel, L., and D. Reinberg. Common themes in assembly and function of eukaryotic transcription complexes. *Annu. Rev. Biochem.* **64**:533-561 (1995).
11. Whitelaw, M. L., J.-Å. Gustafsson, and L. Poellinger. Identification of transactivation and repression functions of the dioxin receptor and its basic helix-loop-helix/PAS partner factor Arnt: inducible versus constitutive modes of regulation. *Mol. Cell. Biol.* **14**:8343-8355 (1994).
12. Li, H., L. Q. Dong, and J. P. Whitlock. Transcriptional activation function of the mouse Ah receptor nuclear translocator. *J. Biol. Chem.* **269**:28098-28105 (1994).
13. Jain, S., K. M. Dolwick, J. V. Schmidt, and C. A. Bradfield. Potent transactivation domains of the Ah receptor and the Ah receptor nuclear translocator map to their carboxyl termini. *J. Biol. Chem.* **269**:31518-31524 (1994).
14. Ma, Q., L. Q. Dong, and J. P. Whitlock. Transcriptional activation by the mouse Ah receptor: interplay between multiple stimulatory and inhibitory functions. *J. Biol. Chem.* **270**:12697-12703 (1995).
15. Wu, L., and J. Whitlock Jr. Mechanism of dioxin action: receptor-enhancer interactions in intact cells. *Nucleic Acids Res.* **21**:119-125 (1993).
16. Wright, A. P., I. J. McEwan, K. Dahlman-Wright, and J.-Å. Gustafsson. High level expression of the major transactivation domain of the human glucocorticoid receptor in yeast cells inhibits endogenous gene expression and cell growth. *Mol. Endocrinol.* **5**:1366-1372 (1991).
17. Rowlands, J. C., and J.-Å. Gustafsson. Human dioxin receptor chimera

transactivation in a yeast model system and studies on receptor agonists and antagonists. *Pharmacol. Toxicol.* **76:**328-333 (1995).
18. Whitelaw, M. L., J. McGuire, D. Picard, J.-Å. Gustafsson, and L. Poellinger. Heat shock protein hsp90 regulates dioxin receptor function *in vivo*. *Proc. Natl. Acad. Sci. USA* **92:**4437-4441 (1995).
19. Wright, A. P. H., J. Carlstedt-Duke, and J.-Å. Gustafsson. Ligand-specific transactivation of gene expression by a derivative of the human glucocorticoid receptor expressed in yeast. *J. Biol. Chem.* **265:**14763-14769 (1990).
20. McEwan, I. J., A. P. Wright, K. Dahlman-Wright, J. Carlstedt-Duke, and J.-Å. Gustafsson. Direct interaction of the τ 1 transactivation domain of the human glucocorticoid receptor with the basal transcriptional machinery. *Mol. Cell. Biol.* **13:**399-407 (1993).
21. Lue, N. F., P. M. Flanagan, K. Sugimoto, and R. D. Kornberg. Initiation by yeast RNA polymerase II at the adenoviral major late promoter *in vitro*. *Science (Washington D. C.)* **246:**661-664 (1989).
22. Ptashne, M. How eukaryotic transcriptional activators work. *Nature (Lond.)* **335:**683-689 (1988).
23. Martin, K. J., J. W. Lillie, and M. R. Green. Evidence for interaction of different eukaryotic transcriptional activators with distinct targets. *Nature (Lond.)* **346:**147-152 (1990).
24. Tasset, D., L. Tora, C. Fromental, E. Scheer, and P. Chambon. Distinct classes of transcriptional activating domains function by different mechanisms. *Cell* **62:**1177-1187 (1990).
25. Nikolov, D. B., and S. K. Burley: 2.1Å resolution refined structure of a TATA box-binding protein (TBP). *Nat. Struct. Biol.* **1:**621-630 (1994).
26. Sadovsky, Y., P. Webb, G. Lopez, J. D. Baxter, P. M. Fitzpatrick, E. Gizang-Ginsberg, V. Cavailles, M. G. Parker, and P. J. Kushner. Transcriptional activators differ in their responses to overexpression of TATA-box-binding protein. *Mol. Cell. Biol.* **15:**1554-1563 (1995).
27. Schulman, I. G., D. Chakravarti, H. Juguilon, A. Romo, and R. M. Evans. Interactions between the retinoid X receptor and a conserved region of the TATA-binding protein mediate hormone-dependent transactivation. *Proc. Natl. Acad. Sci. USA* **92:**8288-8292 (1995).
28. Zawel, L., K. P. Kumar, and D. Reinberg. Recycling of the general transcription factors during RNA polymerase II transcription. *Genes Dev.* **9:**1479-1490 (1995).
29. Johansen, F. E., and R. Prywes. Identification of transcriptional activation and inhibitory domains in serum response factor (SRF) by using GAL4-SRF constructs. *Mol. Cell. Biol.* **13:**4640-4647 (1993).
30. Joliot, V., M. Demma, and R. Prywes. Interaction with RAP74 subunit of TFIIF is required for transcriptional activation by serum response factor. *Nature (Lond.)* **373:**632-635 (1995).
31. Pugh, B. F., and R. Tjian. Diverse transcriptional functions of the multisubunit eukaryotic TFIID complex. *J Biol Chem.* **267:**679-682 (1992).
32. Koleske, A. J., and R. A. Young. The RNA polymerase II holoenzyme and its implications for gene regulation. *Trends Biochem. Sci.* **20:**113-116 (1995).
33. Reese, J. C., L. Apone, S. S. Walker, L. A. Griffin, and M. R. Green. Yeast TAFIIS in a multisubunit complex required for activated transcription. *Nature (Lond.)* **371:**523-527 (1994).
34. Poon, D., Y. Bai, A. M. Campbell, S. Bjorklund, Y. J. Kim, S. Zhou, R. D. Kornberg, and P. A. Weil. Identification and characterization of a TFIID-like multiprotein complex from *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **92:**8224-8228 (1995).
35. Ossipow, V., J. P. Tassan, E. A. Nigg, and U. Schibler. A mammalian RNA polymerase II holoenzyme containing all components required for promoter-specific transcription initiation. *Cell* **83:**137-146 (1995).
36. Chen, J. L., L. D. Attardi, C. P. Verrijzer, K. Yokomori, and R. Tjian. Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* **79:**93-105 (1994).
37. Koleske, A. J., and R. A. Young. An RNA polymerase II holoenzyme responsive to activators. *Nature (Lond.)* **368:**466-469 (1994).
38. Kim, Y. J., S. Bjorklund, Y. Li, M. H. Sayre, and R. D. Kornberg. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* **77:**599-608 (1994).
39. Hengartner, C. J., C. M. Thompson, J. H. Zhang, D. M. Chao, S. M. Liao, A. J. Koleske, S. Okamura, and R. A. Young. Association of an activator with an RNA polymerase II holoenzyme. *Genes Dev.* **9:**897-910 (1995).
40. Chiang, C. M., and R. G. Roeder. Cloning of an intrinsic human TFIID subunit that interacts with multiple transcriptional activators. *Science (Washington D. C.)* **267:**531-536 (1995).

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