Potent Transactivation Domains of the Ah Receptor and the Ah Receptor Nuclear Translocator Map to Their Carboxyl Termini*

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Sanjay Jain, Kristine M. Dolwick, Jennifer V. Schmidt, and Christopher A. Bradfield†

From the Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois 60611

The Ah receptor (AHR) is a ligand-activated transcription factor that is structurally related to its dimerization partner, the Ah receptor nuclear translocator (ARNT), and two Drosophila proteins, SIM and PER. All four proteins contain a region of homology now referred to as a PAS homology domain. In addition, the AHR, ARNT, and SIM harbor a basic region helix-loop-helix motif in their N termini, whereas PER does not. Previous mapping studies of the AHR have demonstrated that the PAS domain contains sequences required for ligand recognition, dimerization, and interaction with the 90-kDa heat shock protein. They also have confirmed that the basic region helix-loop-helix domain plays a role in both dimerization and sequence-specific DNA binding. To identify domains involved in transactivation of target genes, we generated chimeras of AHR/ARNT deletion mutants with the DNA binding region of the yeast Gal4 protein, transiently expressed these in COS-1 cells, and monitored their capacity to activate the chloramphenicol acetyltransferase reporter gene under the control of a minimal promoter driven by enhancer elements reconstructed by Gal4. Extensive analysis of these fusions revealed that the AHR and ARNT harbor potent transactivation domains within their C termini. Importantly, the amino-terminal halves of both the AHR and ARNT were found to be devoid of transactivation activity.

The AHR is a ligand-activated transcription factor that regulates the expression of a variety of genes involved in xenobiotic metabolism and is a member of a family of signaling molecules that includes its dimerization partner, ARNT, and two Drosophila proteins, SIM and PER (1-6). SIM is a nuclear protein found in certain midline cells that is involved in the development of neurons, glia, and other cells of the embryonic nervous system (7). PER is a predominantly nuclear protein involved in maintenance of the circadian rhythms of locomotor activity and eclosion (6, 8). These four proteins are characterized by a conserved region encompassing approximately 275 amino acids termed the PAS domain (9, 10). In addition to the PAS domain, the AHR, ARNT, and SIM harbor a bHLH motif similar to that found in many heterodimeric transcription factors such as Myc/Max and MyoD/E2A (11, 12). Interestingly, the PER protein does not have a bHLH domain, and thus may not function directly in DNA recognition or transactivation of responsive genes, but rather as an inhibitor of transcription in a manner similar to the Id proteins in the MyoD system (13). Recent evidence has suggested that both the bHLH and PAS domains encode dimerization surfaces that dictate the interactions between the AHR and ARNT and serve to position the basic regions of the two proteins in a manner that allows sequence-specific binding to DREs upstream of regulated promoters (9, 14, 15). Photoaffinity labeling studies coupled with mutational analysis of the AHR suggest that this signaling pathway is initiated through the stereospecific binding of receptor agonists to a region that maps within the carboxyl-terminal portion of the PAS domain (3, 9, 16).

One interesting characteristic of the AHR is that it is activated by a number of potent agonists that are also widespread environmental contaminants, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin and coplanar polychlorinated and polybrominated biphenyls. Current models of ligand-induced signaling suggest that the AHR is associated with hsp90 in the cytosol (15, 17). Upon ligand binding, the AHR translocates into the nucleus, and in the process hsp90 dissociates. In the nucleus, the transformed AHR heterodimerizes with the predominantly nuclear ARNT protein, and this complex binds to DREs and activates the transcription of target genes (4, 9, 15, 18, 19). The mechanisms by which the AHR-ARNT complex activates gene expression are largely unknown. One possibility is that the complex directly interacts with proteins of the general transcriptional machinery and recruits them into a functional transcriptional complex at the promoter element. Alternatively, the AHR-ARNT complex may function to alter chromatin structure through nucleosome displacement and thus act at a distance to increase the accessibility of regulated promoters to the transcriptional machinery (20). These potential mechanisms suggest that distinct functional domains exist within the AHR-ARNT complex that are involved in the activation of transcription from regulated promoters.

To understand the mechanisms of AHR-regulated gene expression and to add to the domain map of this protein, we set out to characterize those regions of the AHR and ARNT that are required for transactivation of target genes. Through the use of the yeast Gal4 fusion system, we present evidence indicating that the C termini of the AHR and ARNT harbor potent TADs.

EXPERIMENTAL PROCEDURES

General Methods—The PCR was performed with annealing temperatures generally a few degrees below the calculated Tm of the primers (21). Template extension was performed at 72 °C using Taq polymerase and standard core reagents from Perkin-Elmer Corp. Typically, the
amplified products were purified from 0.8% agarose gels and subcloned by standard molecular biology methods (22). The recombinant plasmids were confirmed by a combination of restriction enzyme mapping, DNA sequencing and functional analysis of expressed proteins (22, 23). In all cases, we confirmed that the proteins expressed from our constructs yielded the anticipated mobilities upon SDS-polyacrylamide gel electrophoresis and Western blot analysis (see below). The oligonucleotides used in these experiments are listed in Table 1.

Oligonucleotides (5'-3')—The oligonucleotide used were as follows: OL1, CTTATGACCGGCGCGAAGCTGAC; OL2, GATCCGCATGATGAGCAGCGGCGCCAACATCACC; OL3, GGCGTCGACTGATGAGCAGCGGCGCCA; OL4, OL146, GATTTAGGTGACACTATAG; OL5, OL124, ATAAGAATGCGGCCGCACCCTCAATGTTGTGTCGGG; OL6, OL209, GATTTAGGTGACACTATAG; OL7, OL227, GACATCATATCAGAACTACACTATATCCGAGGAATGTTTG; OL8, OL232, ATAAGAATGCGGCCGCACCCTCAATGTTGTGTCGGG; OL9, OL258, ATAAGAATGCGGCCGCACCCTCAATGTTGTGTCGGG; OL10, OL263, ATAAGAATGCGGCCGCACCCTCAATGTTGTGTCGGG.

**Plasmid Construction**—All Gal4 fusion plasmids were constructed in the pSG424 vector (24). This vector contains the amino-terminal 147 amino acids of the yeast Gal4 protein under the control of the SV40 promoter, followed by a multiple cloning site that allows in-frame cloning of sequences derived from a second cDNA. The plasmid pSG9 was prepared by cloning a universal termination fragment, derived from plasmid pBluescript, into the SacI site of pSG424. The plasmid names correspond to the number of amino acids deleted from either the amino terminus or the carboxyl terminus.

**Gal4-AHR Fusion Constructs**—The plasmids pGAHRN146, pGAHRN1315, pGAHRN409/C1615, and pGAHRN520 were generated from the EcoRI, Kpn1, BglII, and SacI restriction enzyme fragments of the murine AHR derived from the plasmid pAH (9), respectively, and subcloned into the compatible sites of pSG424. The plasmid pGAHRN409/C1615 was generated by cloning the SacI restriction enzyme fragment of pGAHRN146 into the corresponding site of pSG424. To generate pGAHR, a 2.2-kilobase pair Kpn1 fragment of pAH was subcloned into the Kpn1 site of pGAHRN146. To construct pGAHRN520, the pmuAHR (9) was first amplified with OL350 and OL209 using PCR, and the amplified product was subcloned into the Sau3AI site of pBS2 to yield pS203. Then, oligo 316 was PCR amplified with OL180 and OL146 (a vector-specific T7 primer), and the amplified product was subcloned into the NotI site of pGAR. The plasmids pGAHRN5487 were constructed by subcloning the 1.1-kilobase pair SacI fragment of pGAHRN520 into the corresponding site of pGAHRN409/C1615. The plasmid pGAHRN5315 was constructed by deleting the SacI fragment from pGAHRN315. To construct the plasmid pGAHRN5316, the transactivation domain of the herpes simplex virus VP16 protein (26, 27) was PCR amplified with OL321 and OL322 and subcloned into the NotI site of pGAR. The plasmids pGARNTNA487 were constructed by cloning the 1.1-kilobase pair BamHI fragment from plasmid pGAHRN520 into the NotI site of pGAR. The plasmid pGARNTNA516 was constructed by cloning the 1.1-kilobase pair BamHI fragment of pGAHRN520 into the NotI site of pGAR. The plasmid pGARNTNA487 was constructed by cloning the NotI-digested VP16 TAD (see above) fragment into the corresponding site of pGAHRN487. The plasmid pGARNTNA657 was constructed by removal of the 900-base pair Kpn1 fragment from pGARNTNA487.
AHR Transactivation Domain

Fig. 1. 

**A** 

Representative CAT assays and Western blots of selected constructs transfected into COS-1 cells. A, representative CAT assays of extracts from cells transfected with selected Gal4-fusion chimeras. Refer to "Experimental Procedures" for transfections and ligand treatment of the cells (−, without βNF; +, with βNF). Due to high level of activity, extracts from the following plasmids were diluted 10-fold: pGAHRNΔ409, pGAHRC418VP, pGAHRNΔ520, and pGAHRRNA520/CA165. Extracts from the plasmids pGAHRRNA409/CA165 and pGAHRNTNA581 were diluted 20-fold. The last lane is from a separate experiment. B, representative Western blots of selected Gal4 fusion constructs with different antisera. Left, Western blots of selected Gal4-fusion chimeras with the anti-Gal4 antisera. Fifteen microliters of the respective extract was loaded in each lane. The arrows indicate the position of the anticipated protein. Refer to text for discussion of the cross-reacting bands. Middle, Western blot of selected Gal4-AHR chimeras with indicated AHR antisera. Ten microliters of the respective extract was loaded in each lane. The arrows indicate the position of the anticipated protein. Right, Western blot of selected Gal4-ARNT chimeras with ARNT antisera. Fifteen microliters of the cell extract was loaded in each lane. The arrows indicate the antigenic position of the Gal4-ARNT fusion. The other lower molecular mass proteins that are seen are presumably proteolytic products of transfected and endogenous ARNT. The position of the prestained molecular mass markers in kDa is indicated on the left of each panel. See "Experimental Procedures" for description of the antibodies.

alkaline phosphatase (Jackson Immunoresearch) were used at 1:2500 dilution, and blots were developed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolylphosphate reagents.

**RESULTS AND DISCUSSION**

**Strategy**—Although in vitro systems have proven valuable in the mapping of AHR domains involved in ligand binding, DRE binding, dimerization with ARNT, and transformation, such systems are not readily applicable to characterize those regions required for transactivation. Moreover, initial reports have suggested that transient expression systems of the AHR and ARNT with a DRE-driven reporter are limited due to ligand responses of not more than 3-fold (36, 37). Given the difficulties inherent in establishing a complete AHR-ARNT signaling system in vitro and the apparent insensitivity of transient expression systems requiring cotransfection of AHR, ARNT, and DRE constructs, we turned our attention toward the development of a system that would accurately reflect the signaling mechanisms within this pathway. In addition, we looked toward a model system that would allow identification of the TADs of AHR and ARNT in a manner that was independent of their dimerization properties or nuclear localization signals.

One method of mapping TADs that satisfies the above criteria is the well documented Gal4 fusion approach (38–42). In this protocol, regions of the cDNA of interest are fused to the DNA binding domain of the yeast Gal4 protein (amino acids 1–147), and the capacity of these chimeras to drive CAT expression from a minimal promoter downstream of UAS elements is monitored (24). This Gal4 domain consists of a zinc finger motif that directs homodimerization and the sequence-specific binding of the protein to the UAS elements and a potent localization sequence that targets the Gal4-chimera to the nucleus (43, 44). An important aspect of this strategy is that amino acids 1–147 of Gal4 lack a TAD, and thus expression of a reporter gene is dependent on the transactivating properties of the heterologous protein fragment fused to it.

3 S. Jain and C. A. Bradfield, unpublished results.
To characterize the transcriptional activity of the different Gal4-chimeras, each construct was cotransfected into COS-1 cells and assayed for its ability to activate expression from a CAT reporter gene pSG5CAT (29, 42). A representative CAT assay is presented in Fig. 1A. To control for variability in transfection efficiencies between samples, all extracts that were used for CAT assays were normalized to the expression of a cotransfected β-galactosidase control (pCH110). To demonstrate that all constructs were expressed and that their observed molecular weights corresponded to that predicted from their cDNA sequence, extracts were characterized by Western blot analysis using antibodies specific for either Gal4, AHR, or ARNT (Table I, Fig. 1B, and below). Analysis with different antisera was necessitated due to our initial observation that Western blot analysis with the anti-Gal4 antisera resulted in a number of nonspecific bands between 70 and 100 kDa, thus limiting its utility in characterizing the expression of fusion proteins in that range (Fig. 1B).

**Table I**

**Summary of expression and transactivation properties of the Gal4 fusion chimeras used in Figs. 3–5**

<table>
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<th>Construct</th>
<th>Gal4</th>
<th>AHRc</th>
<th>AHR N</th>
<th>ARNTc</th>
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<td>+</td>
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<td>+</td>
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</table>

*Antibody.

To control for variability in transfection efficiencies between samples, all extracts that were used for CAT assays were normalized to the expression of a cotransfected β-galactosidase control (pCH110). To demonstrate that all constructs were expressed and that their observed molecular weights corresponded to that predicted from their cDNA sequence, extracts were characterized by Western blot analysis using antibodies specific for either Gal4, AHR, or ARNT (Table I, Fig. 1B, and below). Analysis with different antisera was necessitated due to our initial observation that Western blot analysis with the anti-Gal4 antisera resulted in a number of nonspecific bands between 70 and 100 kDa, thus limiting its utility in characterizing the expression of fusion proteins in that range (Fig. 1B).

**System Validation and Subcellular Localization**—In an attempt to determine if the transient expression in COS-1 cells could provide an accurate reflection of AHR-ARNT signaling, we performed immunocytochemistry to characterize the subcellular localization and ligand-dependent translocation of these full-length proteins in this system. To this end, we first validated the system by demonstrating that transiently transfected AHR and ARNT appropriately localized in COS-1 cells (Fig. 2, A–D). Our results were similar to those reported previously for Hepa 1c17 cells (19). They demonstrate that transiently transfected ARNT is predominantly a nuclear protein, independent of exogenous ligand, and that the AHR is primarily cytosolic in the absence of ligand and translocates into the nucleus upon ligand exposure.

**Mapping the Transactivation Domain within the AHR**—We first chose to map the TADs within the AHR and then to use this data as a reference for subsequent experiments to determine similar domains in ARNT. Since our previous biochemical and functional studies had defined the boundaries of the ligand binding domain of the AHR to reside between residues 186 and 388, we also chose to examine the impact that ligand has on constructs containing this region. This was accomplished by performing experiments in the presence and absence of the agonist βNF. We observed that all Gal4 chimeras fused to the complete ligand binding domain of the AHR drove CAT expression in a ligand-dependent manner (pGAHR and pGAHRNA166) and that all fusions lacking this domain did not retain ligand responsiveness (Figs. 3 and 4). The ligand responsiveness of these constructs is consistent with previous observations demonstrating the modular nature of the AHR, the independence of the ligand binding domain from surrounding sequences, and the potential for this region to confer ligand responsiveness on a glucocorticoid receptor/AHR chimera (9, 16).

Results from our AHR-Gal4 chimeras indicate that the primary transcriptional activity of the AHR lies within the carboxyl terminus. We observed that truncation of the bHLH and PAS domains (i.e., pGAHRNA315 and pGAHRNA409) yielded ligandreliant transactivation activity that was 7–300-fold greater than the pSG424 control (Fig. 3). The marked activity of pGAHRNA409, compared with pGAHRNA315, is consistent with the fact that this chimera is missing a previously described “repressor” domain that now appears to be identical to the AHR’s hsp90 binding region. This result also adds support to the idea that hsp90 is either a cytosolic anchor or cap that prevents AHR-ARNT dimerization (9, 16). We eliminated the possibility that the amino terminus of the AHR harbors a second TAD by demonstrating that the CAT activity of pGAHRCA516 was identical to the control in these assays. Although Western blots indicated that this chimera was expressed (Table I and Fig. 1B), the lack of transactivation ability seen for this construct could have arisen from improper folding or localization of this chimera. Therefore, we also tested the CAT activity of the pGAHRCA516 construct fused to the potent TAD of the VP16 protein (26). The fact that this VP16 chimera yielded a significant transactivation response suggests that its parent pGAHRCA516 is properly folded and localized and that we have yielded a positive result had a TAD been encoded by its sequence. This result adds support to our conclusion that the AHR’s primary TAD lies in its carboxyl terminus and that the amino terminus of this protein is transcriptionally silent.

Once we had determined that the carboxyl terminus of the AHR harbored a potent TAD, we turned our attention to a more detailed mapping study of this region (Fig. 4). Our results demonstrate that the chimeras pGAHRNA409, pGAHRNA520, pGAHRNA409/C165, and pGAHRNA520/C165 all increase CAT activity more than 300-fold relative to the control, pSG424 (Figs. 1A and 4), with the chimera pGAHRNA520/C165 retaining most of the transcriptional activity. These results led us to conclude that the majority of AHR’s transactivation activity resides between the residues 521 and 640.

**Mapping the Transactivation Domain within ARNT**—The Gal4-ARNT chimeras were designed such that their fusion site was positioned in a manner analogous to the Gal4-AHR chimeras described above. The observation that only the full-length ARNT (i.e., pGARN) and the constructs harboring the carboxyl terminus of ARNT (pGARNITN352 and pGARNITN351/Δ604–697) possessed activity in these assays (12–900-fold greater than the pSG424 control) suggests that the carboxyl terminus of ARNT, like the AHR, also harbors a transactivating sequence (Fig. 5). To eliminate the possibility that additional TADs reside in the amino terminus of the ARNT, we demonstrated that pGARNITCA5-255, 418, and 673 were devoid of activity in these assays (Fig. 5). Although Western blots re-

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1 Also observed by Quong et al. (52).
FIG. 2. Subcellular localization of AHR and ARNT in COS-1 cells using indirect immunofluorescence. Panels a and c show immunocytochemistry of pmuAHR and pSportARNT without βNF (−), and panels b and d show immunocytochemistry with βNF (+), respectively, in transiently transfected COS-1 cells. The primary antibody used to localize the AHR was A-1 (rabbit anti-AHR polyclonal antiserum raised against residues 61-419 of mouse AHR, Ref. 19) and ARNTC for the ARNT (see text for description). In these transient transfections, cells in the background that do not show fluorescence serve as internal negative controls. The Gal4-chimeras of full-length AHR and ARNT showed similar immunocytochemical localization profiles, and the location of the nucleus was confirmed by phase contrast microscopy. The results presented here are representative of several independent experiments.

FIG. 3. Gal4-AHR fusion constructs localize the AHR’s TAD to its carboxyl terminus. A, schematic diagram of amino- and carboxyl-terminal deletion Gal4-AHR fusion constructs and the average of their CAT assay results. The values reported are the average of two to four independent experiments with standard error never greater than 25%. The box marked Gal4 represents yeast Gal4 (1-147 amino acids); vertical bars represent the bHLH; stippled box represents the PAS domain with the “A” and “R” repeats indicated therein with black boxes (2); box with left-to-right diagonal lines represents the glutamine-rich region (Q), and the gray shaded box corresponds to TAD of the herpes simplex virus VP16 protein (VP16). The positions of the PAS, ligand binding domain (9), and TAD are indicated with horizontal bars. -Fold induction, reported in the bar graph on the right, is relative to the control pSG424. Bars with gray diagonal lines represent experiments without βNF, and black bars are those with βNF. Ligand-dependent induction is indicated to the right of the bars when relevant.

revealed that these chimeras were expressed (Table I), the negative results could have been due to improper folding or localization of the fusion protein. To test this possibility, we used the same VP16-TAD fusion strategy described above (e.g. pGAHRNA520/CA165). The fact that the pGARNTNA581 chimera showed marked activity suggests that folding and nuclear translocation of pGARNTNA315 was sufficient and strongly argues against the presence of a TAD within the amino-terminal portion of the ARNT protein.

Characterizing Transactivation Domains of AHR and ARNT—The TADs that have been identified in other transcription factors are commonly classified into three groups: rich in acidic amino acids, rich in glutamine, or rich in a composite of proline-serine/threonine residues (26). Our transactivation analysis of AHR and ARNT revealed that these two proteins harbor strong TADs in their C termini (Figs. 3–5). Given the similar expression levels observed for pGAHRNA520/CA165 and pGARNTNA581, our preliminary estimation is that the TADs in both AHR and ARNT are of similar potency in this system. Sequence comparisons of the AHR and ARNT revealed that these proteins harbor glutamines as the preferred residue in their TADs along with interspersed hydrophobic amino acids (Fig. 6). Glutamine-rich activation domains have been described for Sp1 and SNF-5 (45, 46). For Sp1, a glutamine-rich
AHR Transactivation Domain

FIG. 4. Gal4-fusion constructs to fine map AHR's transactivation region. Left, schematic of various Gal4-AHR deletion chimeras; right, average results from at least two independent experiments of their corresponding CAT induction relative to the control plasmid, pSG424 (standard error never greater than 25%). The inducibility, relative to pSG424, of the constructs spanning the TAD and lacking the intact ligand binding domain was independent of the ligand (βNF) (e.g. pGAHRNA315 and pGAHRN409 in Fig. 3); therefore, CAT induction values for certain constructs reported here are from no βNF treatment group only. For example, the constructs pGAHRNA520, pGAHRN409/CΔ105 and pGAHRN520/CΔ165 had similar activity regardless of the presence of βNF. Refer to Fig. 3 legend for description of the domains and patterns used in the schematic.

FIG. 5. Gal4-ARNT fusion constructs localize ARNT's TAD to carboxy terminus. Left, the schematic of various Gal4-ARNT deletion chimeras; right, average results of their CAT induction relative to the control, pSG424, from at least two independent experiments (standard error never greater than 25%). Refer to Fig. 3 legend for description of the domains and patterns used in the schematic.

Hydrophobic patch has been shown to directly interact with the dTAFI110 factor in Drosophila (47). The mechanism by which the Sp1 and AHR-ARNT TADs activate transcription may be different since Sp1 binding sites are generally proximal to the promoter while DREs tend to be predominantly distal. An additional possibility is that the AHR and ARNT may harbor more complex TADs since each has secondary sequence motifs that may also function in transactivation. For example, AHR residues 521–575 are rich in acidic amino acids (19 out of 54 residues are glutamate or aspartate, Fig. 6). Transactivation domains rich in acidic residues have been shown to act at positions distal from their promoters (48) and appear to be responsible for the activity found in a number of bHLH transcription factors (12). In addition, ARNT's TAD also shows preference for proline/serine/threonine residues (Fig. 6). Domains rich in these residues have been shown to account for the transcriptional activity of factors like c-Myc (49). In Myc, it has been shown that the negative charge acquired by phosphorylation of a serine and threonine residue in this domain is critical for transcriptional activity, and thus such domains may act in a manner similar to TADs rich in acidic residues (50).

The preceding experiments with Gal4 fusions lead us to the following conclusions. First, the only known mammalian AHR:

521 ELPFRNRSGLAPPPTVQGQA
568 RITFIGHTYVQDSNNFNYLNNACQPOQPGHJLSCMLOFRKL
619 CQGLO lorepoloenceyvcomcqpoq

ARNT:

582 AENFRNLSGLAPPTVQGQA
698 CQGLO lorepoloenceyvcomcqpoq
723 SSSSGQVPOQPOQPGHPVTLQVSNYNVEKPDNTPMF
770 OPPF5E

Fig. 6. Comparison of transactivation regions in the C termini of AHR and ARNT. Amino acid sequences (single letter code) of residues 521–640 and 582–774 of AHR and ARNT are shown, respectively. Glutamine rich residues are indicated in boldface, serine/threonine/proline regions are in boldface and underlined, and glutamic and aspartic acid residues are in boldface and double underlined. The numbers on the left correspond to the first residue in that row. The dashes indicate deletion of residues 604–697 corresponding to pGARNTNA581/A604-697.
bH3.1-PAS proteins, AHR and ARNT, harbor potent TADs in their C termini. This observation is consistent with the known transcriptional roles of the AHR-ARNT complex in the regulation of gene expression. Second, sequence analysis of the TADs of the AHR and ARNT demonstrates that their transactivation properties may be related to the presence of glutamine-rich or acidic or serine/threonine/proline residues. The presence of TADs in both members of a known PAS-heterodimeric pair, AHR and ARNT, indicates that some transactivation redundancy exists in this system, such that the TAD of either protein may be sufficient to activate transcription of their target genes or that each may function in unique transcriptional environments. Alternatively, both AHR and ARNT may act synergistically to activate their target genes. This latter hypothesis is supported by the observation that an ARNT construct missing target genes. This latter hypothesis is supported by the observation that an ARNT construct missing

Acknowledgments—We thank Alan Poland for the polyclonal antibodies against the AHR and ARNT and Michael Green and Mark Ptashne for the plasmids pG5BCAT and pSC424, respectively.

REFERENCES


Preliminary observations suggest that the carboxyl-terminal half of SIM (rich in glutamine and serine residues) but not of PER, also harbors strong transactivation activity (S. Jain, K. M. Dolwich, J. V. Schmidt, and C. A. Bradford).