The basic helix-loop-helix PAS (bHLH-PAS) transcription factors SIM1 and arylhydrocarbon receptor (AHR) are involved in the control of feeding behavior. 

SIM1 haploinsufficiency causes hyperphagia in mice and humans, most likely by perturbing the hypothalamus function. The administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a ligand of AHR, causes severe anorexia, which also appears to be of central origin. Both SIM1 and AHR require heterodimerization either with ARNT or ARNT2 to function. Here, we characterize the promoter for Sim1 and show that a consensus AHR-ARNT/2 binding site positively regulates its activity in the context of transfection experiments in Neuro-2A cells. A gel shift assay indicated that AHR-ARNT2 can bind its putative site in the Sim1 promoter. Overexpression of Arnt, Arnt2, or Ahr increased the activity of a reporter construct containing the Sim1 promoter by 1.8-, 1.5-, and 2.2-fold, respectively, but failed to do so when the AHR-ARNT/2 binding site was mutated. Similarly, TCDD increased the activity of the reporter construct by 1.8-fold but not that of its mutated version. Finally, we found that TCDD increased Sim1 expression in Neuro-2A cells and in mouse kidney and hypothalamus by 4-, 3-, and 2-fold, respectively. We conclude that Sim1 expression is regulated by AHR-ARNT/2. This result raises the possibility that Sim1 mediates the effect of TCDD on feeding and points to a complex network of regulatory interactions between bHLH-PAS proteins.

The paraventricular nucleus (PVN) of the hypothalamus contains neuroendocrine cells that are critical for the regulation of several physiological processes, including energy balance and blood pressure. Loss-of-function experiments in mice have shown that the bHLH-PAS transcription factor SIM1 is essential for the differentiation of PVN neurons (1). In the absence of Sim1, virtually all PVN neurons fail to develop. Interestingly, whereas Sim1-homozygous mice die shortly after birth, presumably from the PVN defect, Sim1 heterozygous mice survive and develop hyperphagia and obesity (2). Also, a balanced translocation interrupting SIM1 was found in a child with isolated hyperphagia and severe obesity (3). Since the PVN is a key regulator of appetite and Sim1 is not expressed in other nuclei involved in the control of feeding, it has been proposed that PVN dysfunction causes the hyperphagia associated with Sim1 haploinsufficiency. Such a PVN dysfunction could be related to the developmental function of Sim1. Indeed, the PVN of Sim1 heterozygous mice is hypocellular (2). On the other hand, Sim1 is continuously and strongly expressed in the mature PVN, raising the possibility that it could also control feeding behavior physiologically.

The arylhydrocarbon receptor (AHR), another bHLH-PAS protein, also has the potential of affecting feeding behavior. The subcellular localization of AHR is determined by its interaction with small ligands, which include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a toxic aromatic hydrocarbon produced in our industrial environment. Upon binding to TCDD, AHR is translocated from the cytoplasm to the nucleus where it activates transcription of target genes (reviewed in Refs. 4–6). Administration of a single sublethal dose of TCDD to rodents specifically induces anorexia without causing nausea or triggering other forms of behavior (reviewed in Refs. 7 and 8). Other toxic effects of TCDD include thymic involution, immunosuppression, and various endocrine abnormalities. However, decreased food intake is the cause of the weight loss associated with acute TCDD toxicity and is a major contributor to the lethality observed at higher dose. Mutant analysis has confirmed that TCDD toxicity requires Ahr (9). The mechanism by which TCDD induces anorexia remains unknown, but there are some evidences that it might involve the hypothalamus (reviewed in Refs. 7 and 8).

SIM1 and AHR belong to a group of proteins that need to heterodimerize with members of another group of bHLH-PAS proteins, of which only four representatives are as yet characterized: Arnt (10), Arnt2 (11), Bmal1/Mop3 (12–15), and Bmal2/Mop9 (16–19). SIM1 and AHR each can physically interact with Arnt, Arnt2, or Bmal1/Mop3 to form heterodimers (4, 11, 20). The ability of SIM1 or AHR to interact with Bmal2/Mop9 has not been reported. Mutant studies have established that heterodimerization of SIM1 with Arnt2 is required for PVN development (20–22). Although SIM1 and AHR interact with the same partners, they appear to bind different DNA sequences (23, 24). Systematic investigations of DNA binding specificities of bHLH-PAS proteins have shown that a heterodimer composed of Drosophila SIM or mammalian SIM1 and Arnt preferentially binds to GT/G/A/G/TG, whereas mammalian AHR-Arnt preferentially binds to TNGC/TG, which defines the so-called dioxin-responsive element (DRE) (24–27).

Here, we characterize the Sim1 promoter and show that its...
Fig. 1. Identification of Sim1 transcription start site by 5′-RACE analysis. A, scheme showing the position of the primers used for the 5′-RACE analysis. A RACE fragment generated with primer GSP1 served as a template in nested PCR reactions using either primers GSP2 or GSP3. B, agarose gel showing nested PCR products of 560- and 340-bp amplified with primer GSP2 or GSP3, respectively, from newborn kidney tissue. The size of these fragments and their sequences indicate that the Sim1 transcription start site is located 1121 bp upstream of the translation initiation codon. C, comparison of mouse and human genomic sequences located immediately 5′ of the transcription start site. The identity between the two sequences is 77% over a stretch of 184 bp. Potential TATA, GC, and CAAT boxes are outlined.

activity is positively regulated by AHR-ARNT2 and TCDD in cultured cells as well as in mice. These observations raise the possibility that Sim1 mediates the effect of TCDD on feeding behavior and suggest the existence of complex regulatory interactions between bHLH-PAS proteins.

**EXPERIMENTAL PROCEDURES**

Constructs—Sim1 genomic fragments were generated by PCR from a mouse BAC clone and were inserted into pGL3-basic and pGL3-promoter vector expression systems, which contain a luciferase reporter gene (Promega Corp., Madison, WI). The DRE, located in the Sim1 promoter, was mutated by using the QuikChange Site-directed Mutagenesis Kit (Stratagene). Arnt and Ahr expression vectors were generous gifts from Drs. Yoshihiko Fujii-Kuriyama and Christopher Bradfield, respectively. The Arnt2 expression vector has been described previously (20).

**Chemicals**—TCDD was purchased from The National Cancer Institute Chemical Carcinogen Repository Midwest Research Institute. For cell culture experiments, it was dissolved in Me2SO and added to culture media at a final concentration of 10 nM when 40% confluence was reached (25–31). Cells were harvested after 48 h of incubation. For animal experiments, TCDD was dissolved in corn oil/acetone mix (95%/5%, v/v) and administered intraperitoneally at a dose of 360 μg/kg to 5-month-old C57BL/6 male mice.

**RACE**—Rapid amplification of cDNA ends (RACE) analysis was performed by using the Clontech SMART RACE cDNA amplification kit according to the manufacturer’s protocol. Briefly, first-strand cDNA synthesis was performed using 1 μg of total RNA. Touchdown PCR was then performed with Sim1 gene-specific primer GSP1 (5′-GTCGAGTTGGAGGGGATGGGAGGC-3′). Nested PCR was performed with primers GSP2 (5′-CCGGGAGACACGATGTGGGACAG-3′) or GSP3 (5′-CCGGGAGACAGAGCCTGGCTGGGA-3′). The nested PCR products were cloned into pLivSelect-PCR Cloning vector (Bio S&T Inc., Montreal, Quebec, Canada) and sequenced.

**Cell Transfection and Luciferase Assay**—The Neuro-2A cell line was purchased from American Type Culture Collection (Manassas, VA). Neuro-2A (N2A) cells were maintained in minimum essential Eagle’s medium with 10% fetal bovine serum, 2 mM L-glutamine, and Earle’s balanced salt solution adjusted to contain 1.5 mM sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate. Cells were transfected with Lipofectin (Invitrogen) according to instructions from the manufacturer. Transfection was performed 24 h after seeding 1 × 105 cells/well in 6-well tissue culture dishes using 2.0 μg of plasmid. In co-transfection experiments, 1.5 μg of each plasmid was used. All transfection experiments also included the pBI plasmid (1 μg) as an internal control. After overnight incubation, the medium was changed, and cells were cultured for 48 h. Cells were then scraped from the dishes, pelleted by centrifugation, and resuspended in lysis buffer. Luciferase activity was measured using the Luciferase Assay System (Promega) according to the manufacturer. Aliquots of the lysate were used for assay of β-galactosidase activity to control for transfection efficiency. Luciferase activity was expressed as relative light units compared with that of control vectors and shown as the mean ± S.D. of three independent transient transfection experiments, each performed in duplicate.

**Electrophoretic Mobility Shift Assay**—Mouse AHR, ARNT2, and SIM1 proteins were synthesized in vitro using the TnT-coupled reticulocyte lysate system (Promega). The reactions were carried out for 90 min at 30 °C. The relative amounts of the translated proteins were semi-quantified by visualization on 10% SDS-PAGE after incorporation of biotin-lysyl-tRNA (Promega).

Electrophoretic mobility shift assay was done as described by Swan son et al. (27). Briefly, equal amounts of dimerizing partners were incubated for 2 h at 30 °C in the presence or absence of TCDD (0.4 μM). Poly(dIdC) (200 ng or 1.2 mg) and KCl (final concentration: 100 mM) were added to the protein mixture in a final volume of 10 μl, and incubation was carried out at room temperature for 10 min. In some instances, a 500-fold excess of unlabeled oligonucleotide was also added. End-labeled double-stranded oligonucleotide (100,000 cpm) was then added, and the sample was incubated for an additional 10 min at room temperature. The reaction mixture was submitted to electrophoresis on a 4% acrylamide non-denaturing gel using 0.5× TBE (45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH 8.0) as the running buffer. The gel was dried and autoradiographed. Each electrophoretic mobility shift assay experiment was performed independently three times. The nucleotide sequences of the oligonucleotides used were: D1, 5′-TCGAGCTGGGAGGATATGCTGACATACC-3′; D2, 5′-TCAGAGATGTGAGCAGACATGGCAAGCGGG-3′; M1, 5′-ACGGCAAGCTG-3′; S1, 5′-GCGAGCCCAGTCGCTGGGGGG-3′; S2, 5′-CCCCCCCCCGGATCTGGCTGGCG-3′; M2, 5′-CCCCCCCCCACTGGGCTCGCG-3′. Oligonucleotides D1 and D2 contain a prototypical DRE that was characterized by Swan son et al. (27).

**Reverse Transcription-PCR Analysis**—Total RNA was isolated using TrizPure Isolation Reagent (Roche Diagnostics, Laval, Quebec, Canada). Single-strand cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen). Reverse transcriptase-PCR for detection of Ahr (32), Arnt (33), and Arnt2 (34) expression was performed as described previously.

Primers for real-time PCR were designed using Primer3 software (Whitehead Institute for Biomedical Research), subjected to a melting curve analysis, and then examined by standard PCR and agarose gel electrophoresis for correct product size. Quantitative PCR was performed using a Smart Cycler (Cepheid). For each reaction, 25 μl of SYBR-Green PCR, 1 μl of cDNA, 1 μl of each primer (5 μM), and 12.5 μl of SYBR-Green JumpStart Taq Ready Mix (Sigma) were mixed together. Sim1 PCR was carried out at 94 °C for 15 s, 59 °C for 30 s and, 72 °C for 30 s, for 45 cycles. The same conditions were used to amplify the β-actin with the exception that the annealing temperature was 55 °C. Real-time PCR reactions using the same starting amount of RNA purified from the different tissues studied showed that TCDD did not change the level of β-actin expression. For each sample, reactions were performed in duplicate, and threshold cycle numbers were averaged. Sim1 expression was normalized to β-actin and fold induction was calculated according to the formula described by Buckhaults et al. (35).

The sequences of the primers used were: Sim1, 5′-CCAAATAGGATTTG-3′ and 5′-GGAG TAGATATGAGACCCGAGCTTC-3′; β-actin, 5′-AGGGTACATCGTTAGAAGCCT-3′ and 5′-GGCAGTAATTCTGTTGATC-3′.
RESULTS

A 5′-RACE approach was used to map Sim1 transcription start site (TSS). A first round of amplification using primer GSP1, which sequence is located in the translated region, was followed by a round of nested amplification with either primer GSP2 or GSP3, located in the 5′-untranslated region (Fig. 1A).

Nested PCRs using primers GSP2 and GSP3 generated fragments of 560 and 340 bp, respectively, from murine newborn kidney (Fig. 1B). Fragments of similar size were also obtained from embryonic brains obtained at 12.5 days post-coitus and newborn hypothalamus (data not shown). Sequencing of these nested amplification products indicated that the TSS is located

Fig. 2. Identification of a functional DRE in Sim1 promoter region. A, promoter activity of Sim1 genomic fragments. A 670-bp fragment encompassing the Sim1 transcription start site or various subfragments were cloned upstream of a luciferase reporter gene in the pGL3-basic vector. These different constructs were transiently transfected in N2A cells and the luciferase activity was measured. Relative luciferase activities are expressed as fold induction over the value obtained from transfection of the pGL3-basic empty vector. Results were significantly different when compared between each other (p < 0.01) with the exception of the comparison between vector A4 and the control vector (p > 0.5). B, effect of Sim1 genomic fragments on the activity of the SV40 promoter. Sim1 genomic fragments were cloned upstream of the SV40 promoter and of a luciferase reporter gene in the pGL3-promoter vector. These constructs were transiently transfected in N2A cells. Relative luciferase activities are expressed as fold induction over the value obtained from transfection of the pGL3-promoter vector which contains the SV40 promoter and the luciferase reporter gene but no Sim1 sequence. Comparison between results obtained with vector A4 and the other vectors showed significant difference (p < 0.01), whereas comparison between results obtained with vectors A4m, A6 and the control vectors showed no significant difference (p > 0.05). C, loss of a potential DRE reduced Sim1 promoter activity. Results were significantly different when compared between each other (p < 0.001). Results represent means ± S.D. of three independent experiments, each performed in duplicates. Statistical analysis was performed with a Student t test.
FIG. 3. Binding activity of AHR-ARNT2 to the Sim1 DRE. AHR and ARNT2 were produced by in vitro transcription and translation, and comparable amounts of each protein were incubated with labeled oligonucleotides containing Sim1 DRE (S), mutated Sim1 DRE (M), or a prototypical DRE (D) in the presence or absence of TCDD (0.4 μM), 500-fold excess of unlabeled Sim1 DRE oligonucleotide, or a high concentration of di-DC. The band corresponding to the oligonucleotide heterodimer complex is indicated by an arrow. The experiment was performed three times independently.

Regulatory Interaction between Sim1 and Ahr

1121 nucleotides upstream of the translation start codon. Sequence analysis showed the presence of consensus TATA, GC, and CAAT boxes, respectively, 43, 94, and 133/164 bp upstream of the TSS (Fig. 1C). A high level of identity between mouse and human sequences (77%) was found over a segment of 184 bp located immediately 5’ to the TSS.

We next cloned a 670-bp fragment (named fragment A), encompassing Sim1 TSS, upstream of a luciferase reporter gene in the promoterless pGL3-basic vector (Fig. 2A). This construct was transiently transfected into N2A cells, which express Sim1, and luciferase activity was assayed (Fig. 2A). Fragment A increased luciferase activity over base line by 6-fold, indicating promoter activity. Deletion of 250 bp at the 5’ end of fragment A resulted in a 5-fold increase of luciferase activity, suggesting the existence of an element between positions −15 and +295 that inhibits promoter activity (Fig. 2A, fragment A2). Further deleting a 38-bp segment, which contained a potential TATA box, decreased the activity of the promoter by 60% (fragment A3), whereas a larger 3’ deletion removing the GC and CAAT boxes abolished promoter activity (fragment A4). Deletion of 322 bp at the 5’ end of fragment A was associated with low but detectable promoter activity (fragment A5). This series of deletions locates Sim1 minimal core promoter between position −178 and the TSS. Similar results were obtained with the 293 cell line, which also expresses Sim1 (data not shown).

Fragment A4 had no promoter activity. However, fragment A4 contained a positive regulatory element because it increased the activity of a SV40 promoter by more than 3-fold in N2A cells, as shown by luciferase assay (Fig. 2B). Deletion of 47 bp at the 3’ end of the A4 fragment resulted in a 50% decrease of this stimulatory activity (Fig. 2B, fragment A6). We noticed that the consensus sequence of the DRE (TCGCCGTG) was present in this 47-bp segment. Interestingly, deletion of 4 base pairs within this element (CGGTG) decreased the stimulatory activity of fragment A4 on the SV40 promoter by 50%, strongly suggesting that this potential DRE corresponds to the positive regulatory element mapped to the 3’ end of fragment A4 (Fig. 2B).

Consistently, introduction of the same 4-bp mutation in the A2 fragment (fragment A2m) decreased its promoter activity by 50% (Fig. 2C). Therefore, we identified a potential DRE that positively affects the activity of both SV40 and Sim1 basal promoters.

It has been shown that the sequence flanking a consensus DRE can affect the binding of the AHR-ARNT/2 complex (25–27). To determine whether the AHR-ARNT complex binds the potential DRE found in fragment A, we performed mobility shift studies using in vitro translated AHR and ARNT2. We found that AHR-ARNT2 binds a 26-bp oligonucleotide that contains Sim1 DRE but not its mutated version (Fig. 3). Binding to Sim1 DRE was increased in the presence of TCDD, decreased in the presence of an excess of unlabeled oligonucleotide, and unchanged in the presence of unlabeled mutant oligonucleotide (Fig. 3; not shown).

To further address the contribution of the DRE to the regulation of Sim1 expression, the effect of Ahr, Arnt, or Arnt2 overexpression on the Sim1 promoter activity of fragment A2 was assessed in co-transfection experiments using N2A cells, in which these three genes are expressed (data not shown). Overexpression of Ahr, Arnt, and Arnt2 increased the promoter activity associated with fragment A2 by 1.8-, 1.5-, and 2.2-fold, respectively, but not that of its mutated version (A2m) in which the DRE has been destroyed (Fig. 4A). The addition of TCDD increased by itself the promoter activity associated with fragment A2 by 1.9-fold but had no effect on fragment A2m (Fig. 4B). Transfection of the Ahr expression vector in the presence of TCDD increased the promoter activity of fragment A2 by 2.4 ± 0.1-fold (not shown); this increase was 28% higher than that obtained in cotransfections performed in the absence of TCDD. Although small, this difference is significant (p < 0.05). The lack of an important additive effect of Ahr overexpression and TCDD treatment would suggest that a component of the AHR-ARNT/2 complex or of the Sim1 promoter limits the effect of the DRE element on its activity, at least in the context of the construct used here. Altogether, these results provide functional evidence that AHR-ARNT2 regulates Sim1 promoter.

To explore the regulatory interaction between Ahr and Sim1 in vivo, we studied the effect of TCDD on Sim1 gene expression in N2A cells using quantitative real-time PCR. We observed that the addition of TCDD to N2A cells increased Sim1 mRNA levels by 4-fold compared with untreated cells (Fig. 5C). We next examined Sim1 expression in the kidney and hypothalamus of C57BL/6 mice treated with a single dose of TCDD (360 μg/kg), which has been shown to cause severe hypophagia in mice from this strain (36). We confirmed the efficiency of this dose by observing effects in C57BL/6 mice over a 2-week period (Fig. 5, A and B). We next measured Sim1 expression levels in mice tissues 3 days after injection of TCDD or vehicle. A 3- and 2-fold increase of Sim1 expression in kidney and hypothalamus, respectively, was observed after TCDD administration (Fig. 5C). On the whole, we conclude that Sim1 expression is regulated in vivo by AHR-ARNT/2.

DISCUSSION

AHR mediates at least two distinct sets of responses. First, AHR regulates an adaptive response pathway to environmental contaminants such as the polychlorinated dioxins that contaminate industrial chemicals (4–6). Specifically, AHR upregulates xenobiotic metabolizing enzymes such as CYP1A1 that participate in the degradation and elimination of these toxins. Second, AHR mediates the toxic effects of these compounds. For instance, the administration of TCDD triggers a complex syndrome characterized by thymic involution, immunosuppression, endocrine abnormalities, and anorexia. Although it has been shown that Ahr is required for the toxicity of TCDD, little is known about the downstream molecular events that lead to its effect (9). Our observation that Ahr acts upstream of Sim1 raises the possibility that the latter is involved in mediating the toxicity of TCDD and points to a complex hierarchical interaction between bHLH-PAS proteins.

TCDD and the Control of Appetite—The mechanism underlying the effect of TCDD on feeding remains unknown but
might involve the hypothalamus, in keeping with its central role in the control of appetite (reviewed in Ref. 7). The observation that the intracerebroventricular injection of TCDD triggers an even stronger effect on appetite than when it is administered peripherally supports this possibility (37). Several lines of evidence in fact suggest that $Ahr$ can act in the PVN, a critical hypothalamic center regulating feeding behavior. First, $Ahr$, $Arnt$, and $Arnt2$ are expressed in the mature PVN (2, 38). Second, TCDD administered peripherally can induce the expression of $Cyp1a1$, a direct target of $Ahr$, in the hypothalamus by 6-fold within 1 day suggesting that it crosses the blood-brain barrier (39). Also, TCDD increases the expression of $c-Fos$ in the PVN within 3 days after the administration of a single dose (40). Third, various endocrine perturbations have been observed following the administration of TCDD, some of which were related to processes regulated by the PVN. For instance, chronic administration of TCDD results in an increase of corticotropin-releasing hormone and vasopressin production by the PVN (41). Also, it has been shown that TCDD can potentiate the secretion of oxytocin by PVN cells (42). Our finding that TCDD increases $Sim1$ transcript levels in the hypothalamus further supports the idea that TCDD can modulate gene expression in this structure.

PVN dysfunction appears to underlie the hyperphagia associated with $Sim1$ haploinsufficiency. The fact that the PVN of $Sim1^{-/-}$ mice is hypocellular suggests that this dysfunction is of developmental origin. On the other hand, $Sim1$ is continuously expressed in the PVN postnatally, raising the possibility that it functions physiologically to control feeding. If this were indeed the case, appetite would then be remarkably sensitive to $Sim1$ levels because reduction of its gene dosage by 50% is sufficient to markedly increase food intake. Conversely, the 2-fold increase of its expression level in the hypothalamus following TCDD administration would be predicted to decrease feeding. Thus, it is tempting to speculate that the induction of $Sim1$ expression by TCDD mediates, at least partially, its effect on feeding. Alternatively, $Sim1$ could mediate the effect of TCDD on other aspects of PVN function such as the secretion of hormones. Also, both the loss of AHR function and the administration of a hydrocarbon ligand of AHR disrupt nephrogenesis (43). $Sim1$ is strongly expressed in the developing nephrons, and its expression in the kidney increases upon TCDD administration, as shown by our study. It is thus possible that $Sim1$ plays a role downstream of $Ahr$ in the kidney.

It is unclear whether AHR normally plays a physiological role in the control of feeding behavior in the absence of TCDD. AHR mutant mice were not reported to be thin nor obese, although on some backgrounds, their growth appears transiently decreased (44–46). It will be particularly interesting to determine whether recently identified endogenous ligands for AHR have the same effect on feeding as TCDD and whether they also increase $Sim1$ expression (47–51). The analysis of such natural ligands may reveal some physiological functions of the $Ahr$ pathway that were unraveled by mutant analysis.

**Fig. 4. Effect of Ahr, Arnt, and Arnt2 overexpression or of TCDD supplementation on Sim1 promoter activity.** A, N2A cells were transiently cotransfected with an $Ahr$, $Arnt$, $Arnt2$, or $Sim1$ expression vector and with an expression vector in which the A2 fragment or its mutated version was cloned upstream of the luciferase reporter gene. Relative luciferase activities are expressed as -fold induction over the value obtained from transfection of pGL3-basic empty vector. Results represent the mean ± S.D. of three independent experiments, each performed in duplicate. B, N2A cells were transiently transfected with the pGL3-basic plasmid containing the A2 fragment or its mutated version cloned upstream of the luciferase reporter gene in the presence of TCDD or dimethyl sulfoxide (DMSO). Relative luciferase activities are expressed as -fold induction over the value obtained from transfection of the nonmutated vector in the presence of dimethyl sulfoxide, which was given an arbitrary value of 1. Results represent the mean ± S.D. of three independent experiments, each performed in duplicate.
Regulatory Interaction between Sim1 and Ahr

Fig. 5. Effect of TCDD on weight gain, appetite, and Sim1 mRNA levels. A and B, effect of TCDD administration on weight gain and appetite. Change in weight (in grams) (A) and in total food intake (B) of mice over a 2-week period following a single dose of TCDD (360 mg/kg) or of vehicle (nB/H11021). Results are expressed as -fold induction using the formula of Buckhaults PCR. Results are expressed as -fold induction using the formula of Sim1 expression levels in N2A cells incubated with TCDD or dimethyl sulfoxide and in mice kidney and hypothalamus after administration of TCDD or of vehicle expression levels were measured using real-time PCR. Results are expressed as -fold induction using the formula of Buckhaults et al. (35) after normalization to B-actin levels and represent the mean of independent reactions performed on three N2A cultures and 12 kidney and eight hypothalamic samples. Each of these tissue samples was collected from a different mouse.

regulate target gene expression. First, the activity of two or more bHLH-PAS proteins can be modulated by the availability of a dimerizing partner for which they compete (23, 24, 52). Second, some bHLH-PAS heterodimers, such as HIF-ARNT and Sim1-ARNT, have the potential of binding to the same DNA sequence (24). Third, two bHLH-PAS heterodimers can directly regulate the transcription of the same downstream gene by interacting with distinct regulatory elements as found for AHR-ARNT and HIF-ARNT, which both control the expression of genes coding for other bHLH-PAS proteins.

bHLH-PAS proteins can also regulate their own expression or the expression of genes coding for other bHLH-PAS proteins. For instance, in the fly, SIM positively and directly regulates the promoter. However, we propose that AHR-ARNT2 is not required for basal or tissue-specific expression of Sim1 but provides an additional layer of regulation that modulates Sim1 levels. This mechanism of regulation could be critical for the physiological processes controlled by Sim1 or for TCDD toxicity.

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REFERENCES
1. Michaud, J. L., Rosenquist, T., May, R. N., and Fan, C.-M. (1998) Genes Dev. 12, 3264–3275
2. Michaud, J. L., Boucher, F., Melynk, A., Gauthier, F., Gohu, E., Levy, E., Mitchell, G. A., Himms-Hagen, J., and Fan, C.-M. (2001) Hum. Mol. Genet. 10, 1465–1473
3. Holder J. L., Jr., Butte, N. F., and Zinn, A. R. (2000) Hum. Mol. Genet. 9, 101–108
4. Schiehau, J. V., and Bradfield, C. A. (1996) Annu. Rev. Cell Dev. Biol. 12, 55–89
5. Whitlock, J. P., Jr. (1999) Annu. Rev. Pharmacol. Toxicol. 39, 103–125
6. Gu, Y. Z., Hogenesch, J. B., and Bradfield, C. A. (2000) Annu. Rev. Pharmacol. Toxicol. 40, 519–561
7. Pohjanvirta, R., and Tuomisto, J. (1994) Pharmacol. Rev. 46, 483–549
8. Unkila, M., Pohjanvirta, R., and Tuomisto, J. (1995) Int. J. Biochem. Cell Biol. 27, 443–455
9. Fernandez-Salguero, P. M., Hilbert, D. M., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1996) Toxicol. Appl. Pharmacol. 140, 173–179
10. Hoffman, C., Reyes, H., Chu, F. F., Sander, F., Conley, L. H., Brooks, B. A., and Hankinson, O. (1991) Science 252, 853–856
11. Hirose, K., Morita, M., Ema, M., Minura, J., Hamada, H., Fuji, S., Saijo, Y., Gotoh, O., Sogawa, K., and Fuji-Kuriyama, Y. (1996) Mol. Cell. Biol. 16, 1706–1713
12. Hogenesch, Jl., Chan, W. K., Jackiw, V. H., Brown, R. C., Gu, Y.-Z. G., Pray-Grant, M., Perdew, G. H., and Bradford, C. A. (1997) J. Biol. Chem. 272, 8581–8593
13. Ikeda, M., and Nomura, M. (1997) Biochem. Biophys. Res. Commun. 233, 258–264
14. Takahata, S., Sogawa, K., Kohayashi, A., Ema, M., Minuma, J., Ozaki, N., and Fuji-Kuriyama, Y. (1998) Biochem. Biophys. Res. Commun. 248, 789–794
15. Welting, C. D., and McGlade, C. J. (1998) Mamm. Genome 9, 463–468
16. Hogenesch, J. B., Gu, Y. Z., Moran, S. M., Shimomura, K., Rudliffe, L. A., Takahashi, J. S., and Bradfield, C. A. (2000) J. Neurosci. 20, RC83
17. Maenawa, R., de la Monte, S. M., Chin, M. T., Hay, C. M., Vet, S. F., Perrella, M. A., and Lee, M. E. (2000) J. Biol. Chem. 275, 36847–36851
18. Ohsako, S., Aoki, Y., Nishimura, N., Tohyama, C., Fujii-Kuriyama, Y., and Fujii-Kuriyama, Y. (1998) Biochem. Biophys. Res. Commun. 248, 789–794
19. Takahata, S., Sogawa, K., Kohayashi, A., Ema, M., Minuma, J., Ozaki, N., and Fuji-Kuriyama, Y. (1998) Biochem. Biophys. Res. Commun. 248, 789–794
20. Michaud, J. L., Boucher, F., Melnyk, A., Gauthier, F., Goshu, E., Levy, E., Mitchell, G. A., Himms-Hagen, J., and Fan, C.-M. (2001) Hum. Mol. Genet. 10, 1465–1473
21. Hosoya, T., Oda, Y., Takahashi, S., Morita, M., Kawasahi, S., Ema, M., Yamamoto, M., and Fujii-Kuriyama, Y. (2001) Genes Cells 6, 361–374
22. Keith, B., Adelman, D. M., and Simon, M. C. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 6692–6697
23. Predel, R., Cao, C. M., Tessler-Lavigne, M., and Hankinson, O. (1997) J. Biol. Chem. 272, 4451–4457
24. Woods, S. L., and Whitelaw, M. L. (2002) J. Biol. Chem. 277, 10236–10243
25. Shen, S., and Whitlock, J. P., Jr. (1992) J. Biol. Chem. 267, 6815–6819
26. Umesono, K., and Rosenquist, T., May, R. N., Holder, C. B., Cusanovich, W. A., and Silverstone, A. E. (2002) Toxicol. Sci. 69, 117–124
27. Buckhaults, P., Rapo, C., Stroh, B., and Sen, C. (2001) Mol. Cell Endocrinol. 172, 91–103
28. Jeon, M. S., and Eser, C. (2000) J. Immunol. 165, 6975–6983
29. Fukunaga, B. N., Probst, M. R., Reisz-Porszasz, S., and Hansson, G. O. (1999) J. Biol. Chem. 274, 29270–29278
30. Sim1
31. Lusika, A., Shen, S., and Whitlock, J. P., Jr. (1999) J. Biol. Chem. 268, 6575–6580
32. Swanson, H. I., Chan, W. K., and Bradfield, C. A. (1995) J. Biol. Chem. 270, 26292–26302
33. Wang, F., Samudio, I., and Safie, S. (2001) Mol. Cell Endocrinol. 172, 91–103
34. Kelling, C. X., and Sim1
35. Kudo, T., Naka, S., and Nakagawa, S. (2002) Brain Res. 958, 167–174
36. Shridhar, S., Farley, A., Reid, R. L., Foster, W. G., and Van Vugt, D. A. (2001) Toxicol. Sci. 63, 181–188
37. Pohjanvirta, R., Unkila, M., and Tuomisto, J. (1994) Pharmacol. Biochem. Behav. 47, 273–282
38. Falahatpisheh, M. H., and Ramos, K. S. (2003) J. Neurosci. 23, 1473–1479
39. Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1997) Genes Cells 2, 645–654
Regulatory Interaction between Sim1 and Ahr

47. Song, J., Clagett-Dame, M., Peterson, R. E., Hahn, M. E., Westler, W. M., Sicinski, R. R., and DeLuca, H. F. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 14694–14699
48. Denison, M. S., and Nagy, S. R. (2003) Annu. Rev. Pharmacol. Toxicol. 43, 309–334
49. Adachi, J., Mori, Y., Matsu, S., Takigami, H., Fujino, J., Kitagawa, H., Miller, C. A., III, Kato, T., Saeki, K., and Matsuda, T. (2001) J. Biol. Chem. 276, 31475–31478
50. Wei, Y. D., Bergander, L., Rannug, U., and Rannug, A. (2000) Arch Biochem. Biophys. 383, 99–107
51. Heath-Pagliuso, S., Rogers, W. J., Tullis, K., Seidel, S. D., Cenijn, P. H., Brouwer, A., and Denison, M. S. (1998) Biochemistry 37, 11508–11515
52. Mimura, J., Ema, M., Sogawa, K., and Fujii-Kuriyama, Y. (1999) Genes Dev. 13, 20–25
53. Chan, W. K., Yao, G., Gu, Y. Z., and Bradfield, C. A. (1999) J. Biol. Chem. 274, 12115–12123