Two Murine Homologs of the *Drosophila* Single-minded Protein That Interact with the Mouse Aryl Hydrocarbon Receptor Nuclear Translocator Protein*

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*Drosophila* single-minded, which acts as a positive master gene regulator in central nervous system midline formation in *Drosophila*, its two mouse homologs SIM1 and SIM2, and the mammalian aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) proteins are members of the basic-helix-loop-helix-PAS family of transcription factors. In the yeast-two-hybrid system, we demonstrate a strong constitutive interaction of ARNT with SIM1 and SIM2 and fully ligand-dependent interaction of ARNT with AHR. Both the helix-loop-helix and the PAS regions of SIM1 and of ARNT are required for efficient heterodimerization. SIM1 and SIM2 do not form homodimers, and they do not interact with AHR. We also failed to detect homodimerization of ARNT.

The interaction of ARNT with SIM1 was confirmed with *in vitro* synthesized proteins. Like AHR, *in vitro* synthesized SIM1 associates with the 90-kDa heat shock protein. SIM1 inhibits binding of the AHR:ARNT dimer to the xenobiotic response element *in vitro*. Introduction of SIM1 into hepatoma cells inhibits transcriptional transactivation by the endogenous AHR:ARNT dimer. The mouse SIM1-ARNT dimer binds only weakly to a proposed DNA target for the *Drosophila* SIM-ARNT dimer. In adult mice mRNA for SIM1 was expressed in lung, skeletal muscle, and kidney, whereas the mRNA for SIM2 was found in the latter two. ARNT is also expressed in these organs. Thus mouse SIM1 and SIM2 are novel heterodimerization partners for ARNT *in vitro*, and they may function both as positive and negative transcriptional regulators *in vivo*, during embryogenesis and in the adult organism.

Basic-helix-loop-helix (bHLH)
1 proteins constitute a major group of transcription factors involved in neurogenesis, myogenesis and other morphogenetic processes (1). These proteins act as homo- or heterodimers. The basic region and the helix-loop-helix region are responsible for DNA binding and for dimerization, respectively. The DNA-binding consensus motif for most bHLH proteins, 5'-CANNTG-3', is referred to as the E-box. A subgroup of bHLH proteins is characterized by the presence of a juxtaposed stretch of approximately 300 amino acids, termed the PAS region, which contains two degenerate 50-amino-acid direct repeats, termed PAS-A and PAS-B. The PAS region, initially identified in two *Drosophila* proteins, period and single-minded, and the mammalian aryl hydrocarbon receptor nuclear translocator (ARNT), was later found also to be present in the aryl hydrocarbon receptor (AHR) and the hypoxia-inducible factor HIF-1α (2–5). The PAS region can function as a homodimerization interface, as a heterodimerization domain for other bHLH: PAS proteins, and as an interaction domain with non-PAS regions (6–10).

AHR is the only known ligand-activated bHLH transcription factor (11). It binds a variety of carcinogenic and toxic environmental chemicals, including polycyclic aromatic hydrocarbons (*e.g.* benzo(a)pyrene and halogenated aromatic hydrocarbons *e.g.* 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)), and mediates most if not all of the pathological effects of these compounds. In the cytosol of the mouse hepatoma cell line, Hepa-1, the unliganded receptor is found in a complex with two molecules of the 90-kDa heat shock protein (HSP90) and an unknown 43-kDa protein (12). Ligand binding triggers release of the HSP90 and the 43-kDa molecules, translocation of AHR to the nucleus, its heterodimerization with ARNT, and subsequent DNA binding. The DNA target for the AHR:ARNT dimer, the xenobiotic response element (XRE), present in the 5'-flanking sequences of TCDD-inducible genes, acts as a transcriptional enhancer. The consensus XRE sequence for binding the AHR:ARNT dimer, 5'-TNGCGTG-3', is nonpalindromic (13, 14).

Heterodimerization of ARNT with HIF-1α is required for transcriptional up-regulation of several genes in response to hypoxia (5, 15). At high concentrations, ARNT has been demonstrated to form homodimers and to bind the E-box motif 5'-CACGTG-3', suggesting a potential novel mode of regulation within the bHLH:PAS family of proteins (16, 17).

*Drosophila* single-minded (dSIM) is a positive master gene regulator of central nervous system midline formation. Two murine homologs of the dSIM gene have recently been isolated (18, 19). In addition to their expression in the central nervous system, both proteins are also expressed in a variety of other protein; PAS, PER-ARNT-SIM homology region; PCR, polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; XRE, xenobiotic response element; kb, kilobase pair(s).
tissues during development. The expression of SIM2 in adult mice appears to be restricted to muscle, kidney, and lung (19). The human homolog of SIM2 maps to the Down’s syndrome critical region of chromosome 21 and has been implicated as a candidate gene responsible for the abnormal morphological features observed in Down’s syndrome (20–22). A DNA-binding site (central nervous system midline element (CME), 5′-(G/A)T/TAACGTG-3′), which resembles the XRE, was postulated for dSIM when putatively dimerized with another protein, based on sequence analysis of enhancers of genes involved in midline formation and subject to regulation by dSIM (23). A DNA target consensus (5′-GT(G/A)CAGTG-3′) for the dSIM-ARNT heterodimer, slightly different from the CME, was identified by a binding site selection and amplification strategy (24).

Here we investigate the properties of the two murine SIM proteins, SIM1 and SIM2, in conjunction with mouse ARNT and AHR. We demonstrate that both SIM1 and SIM2 can heterodimerize via their helix-loop-helix/PAS regions with ARNT, but not with AHR, and that they do not form homodimers. Furthermore, SIM1 may have a dual role, both negatively affecting AHR-ARNT binding to the XRE and also acting in concert with ARNT as a novel DNA-binding heterodimer.

EXPERIMENTAL PROCEDURES

Plasmids—The Gal4 activation domain vector pGAD424 and the LexA DNA binding domain vector pBTM117C were generous gifts from J. Colicelli (UCLA) (25, 26). pBTM117C was generated by insertion of a 3.5-kb fragment carrying the CAN1 gene (27) into pBTM117.2 PCR primers were synthesized on a ABI PCR-Mate model 391 (Applied Biosystems) or purchased from IDT (Coralville, IL). The yeast two-hybrid fusion constructs ARNTbHLHAB, ARNTbHLHA, ARNTbHLHC, ARNTb, and AHRcC were generated by PCR using AmpliTaq (Perkin-Elmer) with the corresponding ARNT and AHR cDNAs contained in pcDNA1Neo (9, 10) as templates. For ARNTbHLHAB, pcDNA1Neo-ARNTb was used as template. The SIM1 and SIM2 constructs SIM1AC, SIM1bHLHA, SIM1bHLHC, SIM1AB, and SIM2AC were PCR amplified with full-length SIM1 and SIM2 cDNAs as templates. The primer sequences are available upon request. The PCR products were cloned into pCR2 (Invitrogen), excised with SauI and NotI, and transfected into L-GA and pBTM117.

Full-length SIM1 was PCR-amplified using Ultma Polymerase (Perkin-Elmer) and cloned into pcDNA3 (Invitrogen). The addition of the β-globin 5′-untranslated region from pT7βHER, essentially as described previously for AHR (28), resulted in a 4-fold higher levels of protein expression in the TNT T7-coupled rabbit reticulocyte system (Promega). pcDNA/SIM1bHLH, was the same fashion.

Northern Blot Analysis—A mouse multiple tissue Northern blot (Clontech) was probed with a random primed, [32P]deoxythymidine triphosphate-labeled cDNA fragment of the C-terminal part of SIM1 and SIM2 (starting at the codons corresponding to amino acid 392 of SIM1 and to amino acid 393 of SIM2, respectively). Prehybridization, hybridization, and washing conditions were according to the manufacturer’s protocol. The blot was exposed to Biomax autoradiography film (Kodak).

RESULTS

Interactions of ARNT, AHR, SIM1, and SIM2 in the Yeast Two-hybrid System—This system for assessing protein-protein interactions takes advantage of the modular nature of eukaryotic transcription factors. One protein of interest is fused to a DNA binding domain, and the other is fused to a transcriptional activation domain. Dimerization of the two fusion proteins reconstitutes a functional transcription factor that can activate several reporter genes incorporated into the genome of the host cells. Previously, we demonstrated that full in vitro heterodimerization and XRE binding activity was retained by ARNT and AHR constructs lacking their C-terminal halves, indicating that the bHLH-PAS regions were sufficient for ligand-dependent heterodimerization (9, 10). Here we generated con-
results such that the LexA DNA binding domain and the Gal4 transactivation domain were fused with the bHLH-PAS regions of the mouse proteins ARNT, AHR, SIM1, and SIM2 and with several mutant forms of ARNT and SIM1. A full-length mouse SIM1 mammalian expression plasmid and N-terminal deletion construct (SIM1ΔbH1) were also generated (Fig. 1).

The interactions among ARNTbHLHAB, AHRΔC, SIM1ΔC, and SIM2ΔC were quantitated by assaying β-galactosidase activity in the transformed yeast cells (the β-galactosidase gene in the host cells contains several LexA binding sites in its 5’-flanking sequence) (Table I). The cells were either untreated or treated with the AHR agonist BNF. Heterodimerization of ARNTbHLHAB with AHRΔC was ligand-dependent. BNF treatment resulted in a 40- or 60-fold increase in dimerization of these proteins (as measured by β-galactosidase reporter activity after subtracting the background values of the empty vector controls, and depending on the orientation of ARNTbHLHAB and AHRΔC constructs in the two hybrid vectors). Such a marked effect of ligand on ARNT-ARNT dimerization has previously only been observed in mammalian cells expressing the endogenous AHR and ARNT proteins and not in yeast (31–34). ARNTbHLHAB interacted with SIM1ΔC and less strongly with SIM2ΔC, the addition of BNF having no effect in either case. No ARNT homodimerization was observed with the bHLHAB construct. Similar results were obtained with an ARNT derivative HLHAB, identical to bHLHAB except for the absence of the basic region (data not shown). No evidence for homodimerization of AHRΔC or for it interacting with SIM1ΔC or SIM2ΔC was obtained. Finally, no homodimerization of either SIM1ΔC or SIM2ΔC and no heterodimerization of SIM1ΔC with SIM2ΔC was observed. Thus, in the two-hybrid system, AHR, SIM1, and SIM2 each interacted exclusively with ARNT, and each failed to form homodimers, as did ARNT. Similar results were obtained with each pair of proteins regardless of which protein was expressed as a fusion with the Gal4 transactivation domain and which was expressed as a fusion with the LexA DNA binding domain.

The contribution of the bHLH and the PAS region of SIM1 toward heterodimerization with ARNTbHLHAB was also investigated (Table II). No interactions were observed between ARNTbHLHAB and SIM1 constructs containing only the bHLH, bHLHA, or AB regions. Identical results were obtained for SIM1ΔC with the equivalent ARNT constructs. These results indicate that the entire bHLH-PAS regions of SIM1 and ARNT are required for efficient heterodimerization in yeast.

Constitutive, Ligand-independent Heterodimerization of ARNT with SIM1 Occurs in Vitro—To confirm the observed interaction of ARNT with SIM1, the heterodimerization between ARNT and SIM1 was assessed using in vitro expressed proteins. The proteins were synthesized separately in rabbit reticulocyte lysate, either in the presence (SIM1) or absence (ARNT) of [35S]methionine. They were then incubated together, subjected to immunoprecipitation with antibodies to ARNT, and subjected to SDS-polyacylamide gel electrophoresis (Fig. 2A). SIM1 was coimmunoprecipitated with ARNT, both in the presence or absence of TCDD. SIM1 was absent from the supernatant, indicating that the immunoprecipitation was quantitative (data not shown). A small amount of SIM1 was precipitated in an incubation mixture containing preimmune serum, which was comparable with the amount precipitated in the absence of ARNT protein. ARNT antibodies did not coprecipitate significant amounts of the SIM1ΔbH1 construct (data not shown). In a separate experiment, in vitro synthesized SIM1 failed to bind the AHR photoaffinity ligand 2-azido-3-[125I]iodo-7,8-dibromodibenzo-p-dioxin under conditions in which binding to AHR is clearly discernible (Ref. 10 and data not shown). Thus SIM1-ARNT heterodimerization in reticulocyte lysate is constitutive and TCDD-independent and, as expected, requires helix 1 of SIM1.

SIM1 Associates with HSP90—In the absence of ligand, cytosolic AHR is found in a complex with two molecules of HSP90 (12). When AHR is translated in rabbit reticulocyte lysate, it associates with HSP90 present in the lysate (10, 35). When AHR and SIM1 were synthesized in the presence of [35S]methionine and then treated with HSP90 antibodies, AHR and SIM1 were precipitated, whereas the corresponding control IgM did not precipitate the labeled proteins (Fig. 2B). Thus, like AHR, SIM1 complexes with HSP90.

Weak Binding of the ARNT/SIM1 Heterodimer to the CME—The CME has been proposed as the DNA binding site for dSIM putatively associated with another (ARN-like) protein (23). We analyzed the different forms of the consensus CME core sequence, 5’- (G/A)(T/A)ACGTG(A/C)-3’ in separate EMSAs. No ARNT/SIM1/CME complex formation was observed using ARNT and SIM1 synthesized in vitro in either rabbit reticulocyte or wheat germ lysate, with our routine EMSA conditions using buffer A, which contains 200 mM KCl and 50 μg/ml poly(dI-dC)/(dI-dC). Under less stringent conditions using buffer B, which contains 50 mM KCl and 10 μg/ml poly(dI-dC)/(dI-dC), and using increased amounts of radiolabeled oligonucleotide,
weak complex formation was observed for CMEs 1–4 (5′-TGA-GCTGGAGA/AG/AATACGTGAGAGCGGGA-3′), with minor differences observed between them. No binding was observed for CMEs 5–8 (5′-TGA-GCTGGAGA/AG/AATACGTGAGAGCGGGA-3′). SIM1-ARNT binding on CME2 was directly compared with AHR-ARNT dimer binding to the XRE using binding buffer B for both EMSA assays (Fig. 3). Binding to the XRE required both ARNT and AHR, and binding to CME2 required both ARNT and SIM1. SIM1-ARNT-CME2 complex formation was abolished with a 200-fold molar excess of unlabeled CME2 but was not affected by a 200-fold molar excess of a c-AMP response element oligonucleotide; it could furthermore be supershifted by an ARNT antibody (data not shown). The amount of AHR-ARNT-XRE complex formed was 60 times greater than the amount of SIM1-ARNT-CME2, when equimolar amounts of ARNT, SIM1, and AHR were utilized in the EMSA.

**SIM1 Inhibits the DNA Binding Activity of the AHR-ARNT Heterodimer in Vitro**—To investigate the effect of SIM1 on the formation of the AHR-ARNT-XRE complex, EMSA was performed under the high stringency binding conditions using buffer A (Fig. 4A). No binding to the XRE was observed using equimolar amounts of ARNT and SIM1, either in the presence or absence of TCDD. As demonstrated previously, formation of the AHR-ARNT-XRE complex was dependent on ligand. Addition of SIM1 reduced AHR-ARNT-XRE complex formation. A 50% reduction was achieved with an amount of SIM1 equimolar to that of ARNT and AHR, indicating that SIM1 and ligand-activated AHR have similar affinities for ARNT. The addition of SIM1ΔH1, which as indicated above, does not bind ARNT, did not inhibit AHR-ARNT-XRE complex formation (Fig. 4B).

**SIM1 Inhibits Ligand-dependent AHRC Activity in Mouse**

**Yeast two-hybrid interactions between mouse ARNT and mouse SIM1 domains**

Pairs of plasmids were transformed into host strain L40C and plated on selective medium. Three to six individual colonies were grown in liquid selective medium and subjected to liquid culture assays for β-galactosidase activity (units as defined in Ref. 29). Means ± S.D. are shown.

| pGAD425 derivatives | pBTM117C derivatives | pBTM117C derivatives |
|---------------------|----------------------|----------------------|
| No insert | ARNTΔH1/ΔC | AHRΔC | SIM1ΔC | SIM2ΔC |
| ARNTΔH1/ΔC | 0.25 ± 0.07 | 0.16 ± 0.24 | 5.3 ± 0.9* | 1.01 ± 0.13* | 0.27 ± 0.05 |
| + | ND | 1.48 ± 0.30* | ND | ND | ND |
| AHRΔC | 0.25 ± 0.02 | 0.15 ± 0.04 | 0.09 ± 0.06 | 0.08 ± 0.04 | 0.16 ± 0.07 |
| + | 4.12 ± 0.70* | 0.25 ± 0.02 | 0.10 ± 0.05 | 0.06 ± 0.06 | 0.14 ± 0.10 |
| SIM1ΔC | 7.20 ± 0.60* | 0.11 ± 0.06 | 0.06 ± 0.01 | 0.07 ± 0.03 | 0.06 ± 0.01 |
| + | 7.40 ± 0.70* | 0.10 ± 0.01 | ND | ND | ND |
| SIM2ΔC | 0.68 ± 0.14* | 0.16 ± 0.04 | 0.06 ± 0.03 | 0.11 ± 0.08 | 0.09 ± 0.05 |
| + | 0.58 ± 0.09* | 0.14 ± 0.03 | ND | ND | ND |
| no insert | 0.13 ± 0.04 | 0.08 ± 0.03 | 0.05 ± 0.01 | 0.06 ± 0.01 | 0.07 ± 0.01 |
| + | 0.14 ± 0.04 | 0.10 ± 0.05 | ND | ND | 0.05 ± 0.01 |

* Significant difference (p ≤ 0.05) from a corresponding assay using one empty vector (or from each of the two assays using one empty vector when both controls were performed).

**Hepatoma Cells**—The reporter plasmid pMC6.3k contains the upstream regulatory promoter/enhancer region of the rat *CYP1A1* gene linked to the CAT reporter gene. It directs TCDD-dependent CAT activity when transfected into a cell line possessing functional ARNT and AHR. When the SIM1 expression vector pcDNA3/SIM1 was cotransfected along with pMC6.3k into Hepa-1c1c7 cells, which express both ARNT and AHR (9), it abolished TCDD-dependent CAT activity. Neither SIM1ΔH1 nor the parental vector, pcDNA3, affected TCDD-induced CAT activity (Fig. 5A). Thus, SIM1 negatively modulates transcriptional activation of the AHR-ARNT dimer in
Vivo. SIM1 did not impair the constitutive reporter activity of pSV2CAT in Hepa-1 cells (Fig. 5B), illustrating the specificity of the inhibitory effect of SIM1 on AHR-ARNT and indicating that inhibition is not due to an effect on the general transcriptional machinery.

In the Adult Mouse, SIM1 and SIM2 mRNAs Are Expressed in Kidney and Skeletal Muscle, and SIM1 Is Also Found in Lung (Fig. 6)—A multiple-tissue Northern blot of adult mouse was first hybridized with a probe specific for SIM1 (i.e. with its C-terminal half) and exposed to film. This revealed an exceptionally large 9-kb transcript in kidney, skeletal muscle and at a low level in lung, consistent with the mRNA size found in mouse embryo (18). The blot was then hybridized with a probe specific for SIM2. The SIM2 transcript (4 kb in length) was found to be expressed in kidney and muscle. The SIM2 transcript size is consistent with that found by Ema et al. (19).

**DISCUSSION**

bHLH transcription factors can be divided into two major functional groups. Class A proteins are expressed fairly ubiquitously and form heterodimers with class B proteins. Class B proteins form functionally active heterodimers with class A proteins but do not interact with other class B proteins (1). Analogous to E12 and E47, which act as ubiquitous class A heterodimerization partners for many other bHLH proteins, ARNT should be classified as a mammalian “class A” bHLH-PAS protein, which can interact with the “class B” bHLH-PAS proteins AHR, HIF-1α, SIM1, and SIM2. These last proteins behave like conventional class B bHLH proteins in that they only form functional heterodimers with ARNT. However, unlike conventional class B bHLH proteins, which have limited tissue or lineage specificity, some at least of the bHLH-PAS class B proteins appear not to be restricted to certain tissues and/or lineages but are activated by specific exogenous agents (ligand for AHR, hypoxic conditions for HIF-1). ARNT appears to be absolutely required for the signal-transducing activity of all class B bHLH-PAS proteins, since the latter appear to be capable of binding to DNA only when dimerized in a complex with ARNT (the recently described ARNT2 protein (33) might possibly substitute for ARNT in these interactions). The basic region of ARNT conforms well to the consensus for the basic region of other bHLH proteins. In DNA-binding bHLH-PAS protein complexes studied so far, ARNT binds to the invariant 5′-GTG-3′ motif (24, 36). This motif, reminiscent of an E-box half-site, is present in all of the known DNA targets for bHLH-PAS dimers. The more divergent and variable basic regions of the class B bHLH-PAS proteins thus dictate binding to the non-E-box-like region of bHLH-PAS DNA targets.

Previously, we observed no dimerization of ARNT with ARNT bHLHAB in vitro (9). Here we found no significant homodimerization of the bHLHAB or HLHAB constructs of ARNT in yeast. Thus, ARNT appears to be unable to homodimerize, at least at moderate concentrations (9). However,
other investigators have reported homodimer formation for full-length ARNT partially purified from insect and mammalian cells. Furthermore, E-box-mediated transcriptional activation was demonstrated in vivo, when ARNT expression was driven by strong promoters (16, 17). The homodimerization of ARNT observed by these investigators thus occurred at high nonphysiological concentrations and may not occur under normal in vivo conditions. Hirose et al. (34) also failed to detect homodimerization of ARNT in the yeast two-hybrid system. Since we used constructs truncated at the N terminus and at the C terminus, another possible explanation for our results is the requirement of additional sequence(s) outside of the bHLH-PAS region for ARNT homodimerization.

AHR-ARNT heterodimerization in our LexA-based yeast two-hybrid system is fully ligand-dependent and highly inducible. Other groups have also reconstituted the AHR-ARNT signaling pathway with full-length or with chimeric proteins in yeast and found considerably higher background and lower induction levels (32–34), probably due to use of different yeast expression vectors and/or host strains of different genetic backgrounds.

Treatment with ligand for AHR did not increase the SIM1/ARNT or SIM2/ARNT interactions; nor did ligand increase the SIM1/ARNT interaction in the in vitro communoprecipitation assay. The result for the SIM1/ARNT dimer is consistent with the observation that in vitro synthesized SIM1 did not bind an AHR agonist. The signal(s), if any, promoting association of ARNT with SIM1 and SIM2 remain unknown. It is possible, however, that SIM1/ARNT and SIM2/ARNT interactions are ligand-dependent processes and that the relevant ligand(s) is present in yeast, Hepa-1c1c7 cells, and reticulocyte lysate. The observation that SIM1 binds HSP90 in reticulocyte lysate does not preclude the possibility that reticulocyte lysate contains ligand for SIM1, since AHR can bind HSP90 in reticulocyte lysate even when ligand for AHR is added (10). The highly efficient association of SIM1 with ARNT upon expression in reticulocyte lysate suggests that SIM1 probably binds ARNT more efficiently than it binds HSP90. Other investigators have demonstrated binding of Drosophila SIM to human ARNT and rabbit HSP90 (37). Our observations involve interactions between bHLH-PAS proteins that are all mammalian in origin and are therefore of greater biological relevance.

dSIM, a master gene regulator in central nervous system midline formation, acts as a positive regulator of transcription in Drosophila during development (38). It contains in its C-terminal part several potent transactivation domains. Similar transactivation domains have been identified in ARNT and AHR (39, 40). However, the C-terminal halves of both SIM1 and SIM2 do not show any similarity to the Drosophila homolog. Assuming that the proposed CMEs or the consensus sequence identified by the binding site selection strategy are the DNA targets for the SIM1-ARNT heterodimer, the discrepancy between the strong SIM1-ARNT heterodimerization and its very weak binding activity on the CME could indicate that DNA binding but not heterodimerization of SIM1 with ARNT requires a factor(s) or a post-translational modification activity nearly absent from reticulocyte lysate. Alternatively, there may be cooperative binding of SIM1-ARNT dimers to different CME sites or cooperative binding of SIM1-ARNT with transcription factors that bind to other sites in the flanking region of SIM1-regulated genes. Finally, it is possible that the weak DNA binding in vitro reflects the true in vivo situation. The basic region of SIM1 (and SIM2) starts with its amino-terminal amino acid. AHR requires additional amino acids amino-terminal to its basic region for strong binding to the XRE (28, 41). The absence of equivalent amino acids in SIM1 could explain its very poor DNA binding activity.

Our demonstration that SIM1 can inhibit AHR-ARNT binding to the XRE and can inhibit expression from an XRE-driven reporter gene indicates that SIM1 may act as negative regulator of transcription as well as a positive regulator. The above inhibitory effects may result from SIM1 competing with AHR for binding to ARNT, although we cannot exclude the possibility that SIM1 may also have other inhibitory effects of AHR-ARNT activity. In a similar fashion, SIM1 may act as a negative regulator of all ARNT-dependent genes.

In the adult mouse, the mRNAs for SIM1 and SIM2 are found in tissues, where ARNT and AHR mRNA expression have previously been detected (42). Studies on the embryonic expression patterns of ARNT, AHR (43, 44), SIM1, and SIM2 (17, 18) in the mouse indicate that these proteins are also coexpressed in a number of developing tissues, including portions of the forebrain, embryonic muscle, and facial cartilage. Importantly, during embryogenesis, the distribution of SIM2 expression was always accompanied with that of ARNT (19). This apparent coexpression implies that the interaction between ARNT and the two mouse SIM proteins demonstrated here is likely to be physiologically relevant and that SIM1 and SIM2 are tissue-specific modulators of AHR-ARNT activity.

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Addendum—While this manuscript was being prepared, Ema et al. (19) reported on the isolation and characterization of a cDNA for mSIM. The mSIM cDNA sequence was identical to SIM2 over the bHLH-PAS region but differed in its C-terminal half from it. The corrected SIM2 cDNA sequence has meanwhile been published as an erratum (18). The results and the conclusions drawn from the current study are unaffected, since all expression constructs in our experiments used only the bHLH-PAS region of SIM2 (i.e. SIM2AC), the cDNA sequence of which is identical to that of mSIM.

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