Hypoxia plays a key role in the pathophysiology of many disease states, and expression of the retinoic acid receptor-related orphan receptor α (RORA) gene increases under hypoxia. We investigated the mechanism for this transient hypoxia-induced increase in RORA expression. Reverse transcription-coupled PCR analysis revealed that the steady-state level of mRNA for the RORA isoform, but not the RORα isoform, increased in HepG2 cells after 3 h of hypoxia. Transient transfection studies showed that the hypoxia-induced increase in RORA mRNA occurs at the transcriptional level and is dependent on a hypoxia-responsive element (HRE) located downstream of the promoter. A dominant-negative mutant of hypoxia-inducible factor-1α (HIF-1α) abrogates the transcription activated by hypoxia as well as the transcription activated by exogenously expressed HIF-1α, demonstrating the direct involvement of HIF-1α in the transcriptional activation. However, HIF-1 alone was not sufficient to activate transcription in hypoxic conditions but, rather, required Sp1/Sp3, which binds to a cluster of GC-rich sequences adjacent to the HRE. Deletion of one or more of these GC boxes reduced or eliminated the HIF-1-dependent transcription. Together, these results suggest that the hypoxia-responsive region of the RORA promoter is composed of the HRE and GC-rich sequences and that the transcriptional activation under hypoxia is conferred through the cooperation of HIF-1 with Sp1/Sp3.

Members of the nuclear receptor superfamily play intrinsic roles in a variety of cellular processes, such as embryogenesis, cell differentiation, cell growth, and cell homeostasis (1, 2). Nuclear receptors, as ligand-dependent transcription factors, activate or repress transcription by binding directly to target genes. They are divided into three groups on the basis of their DNA recognition elements. The first group includes the receptors for steroid hormones, including glucocorticoid and androgen, and recognizes an inverted repeat of an AGAACA half-site motif. The second group, which includes the receptors for non-steroid hormones such as thyroid hormone, retinoids, and vitamin D, recognizes a direct repeat of an AGGTCA half-site motif. Most of the nuclear receptors so far identified belong to this group. In contrast to these two groups, the third group includes nuclear receptors that recognize and bind to the AGGTCA half-site motif as a monomer. Little is known about ligands for the members of this group.

The RORα subfamily, which has three members (RORA, RORβ, and RORγ), belongs to the monomeric nuclear receptor subgroup (3). Recently, evidence has been accumulating that RORA is involved in cell differentiation and proliferation. The homozygous mutant mouse staggerer (sg) was initially described as ataxic due to the presence of massive neurodegeneration in the cerebellum. The identification of the widely expressed RORA gene as the site of mutation in the sg mouse has led in recent years to great progress in understanding the molecular basis of its phenotype (4). RORA knock-out mice display the same cerebellar atrophic phenotype as the sg mouse (5, 6). Detailed analyses of such RORα-deficient mice have revealed a number of additional phenotypes outside the nervous system. These include a greater susceptibility to atherosclerosis (7), immunodeficiencies linked to the overexpression of inflammatory cytokines (8, 9), abnormalities in the formation and maintenance of bone tissue (10), changes in muscle differentiation (11), and an increase in ischemia-induced angiogenesis (12). RORα has also been suggested to play a role in vascular function (13, 14). It thus appears that RORα has direct links to a number of age-related pathologies of great medical interest.

RORA is composed of four isoforms that share common DNA and ligand binding domains but differ in their N-terminal regions (15–18). They are produced from a single gene by way of different promoters. It has been suggested that the N-terminal region of RORA affects the specificity of recognition by RORA-responsive elements (21). Although RORA is expressed ubiquitously in vivo, each isoform shows a tissue-specific distribution (18–20). Several tissues or cell types, including liver, brain, endothelial cells, and smooth muscle cells, express mainly RORα1 and RORα4, whereas RORα2 and RORα3 are expressed abundantly in testis. These observations suggest that each RORA isoform plays a different role in a range of cellular processes. Although little is known about the regulation of RORA expression, it has recently been demonstrated that expression of RORA is transiently increased when ischemia is induced in the hindlimbs of mice (12) and in various cell types in response to oxygen deprivation (hypoxia) (20, 22).

Hypoxia is an essential developmental and physiological stimulus that also plays a key role in the pathophysiology of cancer, heart attack, stroke, and other major causes of mortal-
During normoxia, HIF-1α protein is regulated by the level of oxygen (26–28). During normoxia, HIF-1α protein is regulated by the level of oxygen (26–28). Hypoxia-induced activation of the RORα promoter appears to be regulated through a functional interaction between HIF-1 and Sp1/Sp3.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Induction of Hypoxia**—HeLa cells and HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Invitrogen). Caco2 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Invitrogen). Caco2 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Invitrogen).

**Total RNA was prepared using the ISOGEN reagent (Wako, Japan) according to the manufacturer’s protocol. Reverse transcription was performed at 37 °C in a total volume of 20 μl containing 25 mM Tris/HCl, pH 7.8, 37.5 mM KCl, 1.5 mM MgCl₂, 20 μg/ml random primers (Takara Japan), 0.5 μg each dNTP, 10 μM dithiothreitol, 20 units of RNase inhibitor (Takara Japan), 200 units of Moloney murine leukemia virus reverse transcriptase (MBI Fermentas), and 1 μg of total RNA. After 60 min of incubation, an aliquot of the synthesized cDNA was directly amplified in a total volume of 20 μl containing 20 mM Tris/HCl, pH 7.5, 50 mM KCl, 1.5 mM MgCl₂, 1 μM primers, 0.25 mM each dNTP, and 0.25 unit Taq polymerase (Takara Japan). The reaction contained three primers, Po1 (RORα-specific 5’ primer, 5’-AACATGGAGGTCGACTCCGGC-3’), Po4 (RORα-specific 5’ primer, 5’-TGTATCAGCCGACCAGTAGAAG-3’), and Pe (3’ primer common for RORα and ROR4, 5’-GGGTTACAACGTGTCCTCCTG-3’). PCR conditions included 1 cycle at 94 °C for 2 min, 24–32 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s, and 1 cycle at 72 °C for 5 min. The amplified DNA were analyzed by electrophoresis in a 1.5% agarose gel. As a control, the same cDNAs were subjected to PCR (20 cycles) with β-actin primers (5’ primer, 5’-CCATGATCCTGATGAC-3’, 3’ primer, 5’-AAGGACAGGGATCCGGAT-3’). The reverse transcription-coupled PCR experiment was repeated at least three times.

**Plasmid DNA**—A mouse genomic library was screened using the 5’-end of the RORα cDNA as a probe. About 6 kilobases of the XhoI fragment containing the RORα promoter region was subcloned into the XhoI site of the pGKB luciferase vector (Nippon Gene, Japan), in which the HindIII site has been disrupted (pRORαXHLuc). 5’-Deletion mutants at least the 5’ end of the promoter site were constructed by exonuclease HIISI nuclear digestion of pRORαXHLuc at the HindIII site followed by digestion with NheI. After ligation with an EcoRI linker (5’-CGGATCCGG-3’), the larger DNA fragment was isolated and self-ligated. For construction of 3’ deletion mutants, pRORαXHLuc was constructed by removing the HindIII–NheI fragment from pRORαXHLuc by digestion with exonuclease HIISI nuclear digestion of pRORαXHLuc at the HindIII site followed by digestion with NheI. After ligation with an EcoRI linker (5’-CGGATCCGG-3’), the larger DNA was isolated and subcloned into the Smal (changed to an EcoRI site with the linker) and Smal sites of pGV2B. An internal deletion mutant was constructed by combination of the 5’ and 3’ deletion mutant. A HRE-mutated RORα reporter was constructed using the GeneEditor in vitro site-directed mutagenesis system (Promega). The primer used to introduce a mutation within the HRE was 5’-CGCCGCTTCCGGCGACCGGTACACGTTGAGGG-3’. An expression vector for HIF-1α (pBoshHIF-1α) was kindly provided by Dr. Y. Fujii. An expression vector producing a dominant negative HIF-1α (amino acids 1–363, pHIF-1αDN) was constructed by partial digestion of pBoshHIF-1α with Age I.

**Transfection of Cells**—Transfection was performed using the Effectene reagent (Qiagen) according to the manufacturer’s protocol. Briefly, cells were seeded in a 24-well microplate at a density of 4–6 × 10⁴/well 1 day before transfection. Fifty nanograms of reporter DNA, 50 ng of effecter DNA, and 50 ng of pSG5Gal were used for each well. The total amount of DNA was adjusted to 200 µg with pUC19 DNA. After transfection, cells were washed once with phosphate-buffered saline and then grown for 24 h in fresh medium. All transfections were carried out in duplicate, and each experiment was repeated at least three times. The relative luciferase activity was calculated by normalization to the β-galactosidase activity.

**Electromobility Gel Shift Assay (EMSA) and DNase I Footprinting Analysis**—Nuclear extracts from normoxic or hypoxic cells were prepared as described previously (30). The nuclear proteins was incubated in a 10-μl reaction containing 25 mM HEPES, pH 7.9, 50 mM KCl, 1% glycerol, 1 mM dithiothreitol, 0.1% Nonidet P-40, and 50 µg/ml of poly(dI-dC). After 10 min on ice, 32P-labeled probe was added (10,000 cpm/reaction), and the reaction was further incubated on ice for 20 min. The DNA-protein complex was separated in a 5% polyacrylamide gel. For DNase I footprinting, the binding reaction was carried out in a total of 20 µl under the same conditions as EMSA. After binding for 20 min, CaCl₂, MgCl₂, and DNase I were added to final concentrations of 0.25 mM, 5 mM, and 100 ng/ml, respectively. Digestion was performed for 1 min at room temperature and stopped by the addition of a final concentration of 10 mM EDTA. The DNA was then purified and analyzed using a 5% denaturing sequence gel. Oligonucleotides used in the binding experiments were as follows: HREα, 5’-C CGCGAGGCTCACGTCAGT-3’; HREβ, 5’-CGCCGAAGCTCATACGCTGAG-3’; HREpo, 5’-CTAGGAGTAGCTGGAGCTG-3’; GC-box, 5’-ATTCGATCGGCGGCGGAGCG-3’; GA-box, 5’-TACCTGGGGGAGGTGATG-3’; GBE, 5’-AGTTATGTTCAACACTGCTTTAACA-3’.

**RESULTS**

**Induction of ROR4 Isoform Expression by Hypoxia**—During hypoxia, HepG2 cells have been shown to express increased levels of RORα (20). To confirm this in our hands, we first used reverse transcription-coupled PCR to analyze the effects of hypoxia on the steady-state levels of RORα and ROR4 mRNA in HepG2 cells. To avoid artifacts due to fluctuations in the PCR reactions, amplification of both transcripts was carried out in the same reaction using two 5’ primers specific for each isoform and a common 3’ primer. Each 5’ primer selectively amplified its target transcript (data not shown). The amount of RORα mRNA increased several-fold after the cells were exposed to hypoxia for 3 h, whereas that of ROR4 mRNA did not change (Fig. 1). In addition, the increased level of ROR4 mRNA decreased to the control level when the cells were reoxygenated (data not shown). Thus, hypoxia specifically increased expression of the ROR4 isoform in HepG2 cells.

**Transcriptional Regulation of the RORα Gene Promoter by Hypoxia**—Because each RORα isoform differs from the other isoforms only in its N-terminal region, it is likely that the hypoxia-induced increase of ROR4 mRNA expression is regulated at the transcriptional level. To examine this, we analyzed the effect of hypoxia on ROR4 promoter activity in a transient transfection assay. We constructed a reporter DNA in which a fragment encompassing the region from –1015 to +408 of the mouse ROR4 promoter was linked upstream to the firefly luciferase gene. As shown in Fig. 1C, transcription of the ROR4 promoter increased several-fold upon exposure of the transfected HepG2 cells to hypoxia. A similar increase in the promoter activity was also observed when cells were grown in medium containing 100 μM CoCl₂ (Co²⁺), which is known to mimic hypoxic conditions. Additionally, almost identical transcriptional responses to hypoxia were observed both in HeLa
and Caco2 cells, each of which differently expresses RORα4 mRNA (data not shown). These results clearly indicate that the hypoxia-induced increase of RORα4 expression occurred at the transcriptional level. Because the promoter activity of RORα4 was highest in Caco2 cells (data not shown), we used these cells for the following transfection experiments.

To identify cis elements responsible for the hypoxia-induced activation of the RORα4 promoter, we constructed a series of mutant DNAs lacking the 5′-upstream or 3′-downstream region and analyzed their promoter activities under hypoxia (Fig. 2). Although deletion of the region upstream from −397 resulted in a decrease of the basal transcription to about 40% that of the control, any deletion of the 5′-flanking region did not affect the activation of transcription by hypoxia. Deletion of a region downstream from +273 also did not affect the hypoxia-induced activation of transcription, but further deletion to +241 resulted in a loss of responsiveness to hypoxia. An identical result was obtained in the presence of Co2⁺ (data not shown). Inspection of the nucleotide sequence from +240 to +273 revealed the existence of a 5′-ACAGTAC-3′ sequence, which conforms well to the consensus HRE, 5′-(C/G/T)ACGT(G/C)(G/T) (Fig. 3A). To determine whether the putative HRE
found within the RORα4 promoter is indeed responsible for the transcriptional activation under hypoxia, a specific mutation was introduced within the sequence, and its effect on transcription was analyzed. Fig. 3B shows that the hypoxia-induced activation of transcription was markedly abrogated by the mutation within the HRE, indicating its direct involvement in the activation of the RORα4 promoter under hypoxia. Similar results were obtained in the presence of Co^{2+} (data not shown).

Direct Involvement of HIF-1 in the Regulation of the RORα4 Promoter—Activation of hypoxia-inducible genes is mediated in part by the binding of HIF-1 to the HRE. HIF-1α contains two transcriptional activation domains in its C-terminal half (31). We constructed a mutant form of HIF-1α lacking the C-terminal region from amino acid residue 393, which we used as a dominant negative form (HIF-1αDN). The hypoxia-induced activation of the RORα4 promoter was almost completely inhibited by HIF-1αDN (Fig. 3C). The transcription activated by the wild-type HIF-1α was also repressed efficiently in the cells cotransfected with HIF-1αDN under normoxia.

Fig. 4A shows proteins binding to the region containing the HRE. Several nuclear proteins from normoxic and hypoxic cells showed identical patterns of DNA binding. Cold probe DNA competed completely with the binding of all of the proteins, but a specific oligonucleotide containing the HRE failed to compete with the binding of any protein. However, an EMSA experiment carried out using the HRE oligonucleotide showed that proteins bound specifically to the HRE are induced under hypoxia. An excess amount of the cold wild-type HRE successfully competed with the binding, but a mutated HRE did not, demonstrating the specificity of the binding. Additionally, the HRE-binding protein induced by hypoxia disappeared quickly after reoxygenation (data not shown). Another protein, which is present in both normoxic and hypoxic cells, was also found to bind specifically with the HRE. It is not clear at present whether this constitutive protein might be activating transcription factor or CREB (cAMP-response element-binding protein), as has been demonstrated previously (32).

To further confirm that HIF-1 binds to the RORα4 HRE, we performed a DNase I footprinting analysis using a probe from -40 to +341 with nuclear proteins from normoxic or hypoxic cells (Fig. 4C). Consistent with the result obtained in the EMSA experiments, no protein binding to the HRE region was detected with low amounts of nuclear proteins from either normoxic or hypoxic cells. However, the HRE did bind to protein when an increasing amount of the nuclear proteins was used, and the pattern differed between the hypoxic nuclear proteins and the normoxic nuclear proteins in that the HRE region protected by the normoxic nuclear proteins was relatively broad. Together with the results obtained in the transfection experiments, these observations indicate that the HIF-1 induced by hypoxia binds to the HRE and activates the transcription of the RORα4 promoter.

**HIF-1-induced Activation Requires Three GC-rich Elements**—Hypoxia-inducible genes often require cis elements in addition to the HRE for their efficient activation by hypoxia (33–36). In addition to the HRE, three other regions (Box1, Box2, and
Box3) bound with protein(s) that are abundant and expressed constitutively in both normoxic and hypoxic cells (Fig. 4, A and C). The region between +160 and +250 is rich in GC and contains several GC-box-like sequences (Fig. 5), including a typical GC-box at +210, two GGGAGG (GA-box) sequences at +216 and +234, and three GGTTGGG sequences at +171, +191, and +239. To address whether these three Boxes are involved in the hypoxia-induced activation of the RORα promoter, we analyzed the effects of their deletion on the HIF-1-dependent transcription. As shown in Fig. 6, deletion of Box3 (from +223 to +243) reduced the HIF-1-dependent transcription to about 50% of that seen with the wild-type DNA. Deletion of the region from +202 to +243 (deletion of the Box2 and Box3) resulted in a marked reduction of the transcription to about 20% of the control. A similar reduction in transcription was observed in a mutant lacking the region between +155 and +227 (deletion of the Box1 and Box2). In contrast, only a 30% reduction of the HIF-1-dependent transcription was detected on a mutant lacking the region from +155 to +215 (deletion of Box1 and Box2). Although the deletion from +155 to +215 removed both the GC-box and GA-box within the Box2, the ligation of an EcoRI linker created a new GGGAGG sequence in this reporter construct. The Box1 appeared to be dispensable, because deletion of the region between +155 and +178 did not significantly affect the HIF-1-dependent transcription.

**Binding of Sp1 Family to the GC-rich Regions Adjacent to the HRE**—Because the proteins bound to the three GC-rich boxes were constitutively expressed and appeared to be abundant (Fig. 4C), it is highly likely that they are Sp1 and/or its related proteins. To examine this, we performed competitive protein binding experiments with the same DNA probe used in the EMSA (shown in Fig. 4A). As shown in Fig. 7, among several protein-DNA complexes, at least three complexes disappeared specifically in the presence of an oligonucleotide containing either a GC-box or a GA-box. Moreover, these three complexes were recognized specifically by antibodies for either Sp1 or Sp3. The identical protein-DNA complexes were also detected with the hypoxic nuclear proteins (Fig. 4A and data not shown).

The competitive binding of proteins was also analyzed using the hypoxic nuclear proteins in a DNase I footprinting experiment (Fig. 7C). Protein binding at all four sites was competed out by the addition of cold probe DNA, whereas the GC-box oligonucleotide competed only for the protein binding at Box2 and Box3 but not at Box1 and the HRE. The same results were observed with competition experiments using the GA-box oligonucleotide (data not shown). In contrast, the HRE oligonucleotide specifically inhibited HIF-1 binding at the HRE site.

**DISCUSSION**

In the present study we showed that the promoter of the RORα isoform is activated in response to hypoxia. We identified a hypoxia-responsive region downstream of the transcription initiation site that consists of an HRE and several GC-rich sequences. The HRE alone was not sufficient for the efficient activation of transcription of the RORα promoter by hypoxia; the activation was also dependent on at least two GC-rich sequences within Box2 and Box3 to which Sp1/Sp3 bind. The typical GC-box within Box2 appears to be the most important for the transcriptional activation. In contrast, the GC-box oligonucleotide did not compete for the protein binding to Box1, and no protein binding was observed at the GGTTGG sequence at +191, indicating that Sp1/Sp3 does not bind to Box1. The characteristic features of the hypoxia-inducible region within the RORα promoter shares similarities with another hypoxia-inducible gene, carbohydrate IX, in which the Sp1 family in concert with HIF-1 has been demonstrated to fully activate transcription (37). It is known that a functional and/or physical interaction between HIF-1 and another transcription factor is crucial for the transcriptional activation of the hypoxia-inducible genes. Several transcription factors are known to interact with HIF-1, including activating transcription factor/CREB-1 (cAMP-response element-binding protein) in the lactate dehydrogenase A gene (33, 34), AP-1 in the VEGF gene (35), and HNF-4 in the erythropoietin gene (36). Together with the results obtained in the transfection experiments, these observations strongly suggest that the binding of Sp1 and/or Sp3 to at least two GC-rich sequences within Box2 and Box3 is essential for the HIF-1-dependent transcription of the RORα promoter. Our results, therefore, add members of the Sp1 family as another set of transcription factors cooperating with HIF-1 to achieve a high level of hypoxia-induced gene transcription.

The precise molecular mechanism of the interplay between HIF-1 and the Sp1 family is not known at present. The Sp1 family contains ubiquitously expressed transcription factors...
that frequently work in concert with other sequence-specific transactivators to control inducible promoters (38–41). Functional and physical interactions between Sp1 and such factors often result in the synergistic activation of specific target promoters. Mutation of the HRE did not affect the basal transcription of this promoter under normoxia (data not shown), suggesting that the constitutive protein that specifically binds with the HRE (see Fig. 4B) might not be involved in regulating its transcription. Additionally, because deletion of any of the GC-rich sequences also did not result in a decrease in basal transcription (see Fig. 6), Sp1/Sp3 alone might not be involved in the transcriptional activation of this promoter under normoxia. Therefore, Sp1/Sp3 may help recruit HIF-1 to the promoter and/or may serve to better link the action of HIF-1 to the basal transcription machinery.

Initiation of transcription in eukaryotic cells is a complicated multistep process involving a large number of cofactors that act in the remodeling of chromatin and/or the recruitment of RNA polymerase II to the promoters of target genes (42, 43). Of a dozen cofactors, CBP/p300 is a multidomain transcriptional cofactor that acts in conjunction with other factors to regulate transcription. Several lines of evidence show that HIF-1α recruits and interacts physically with CBP/p300 at its C-terminal transactivation domain (31, 44–49). In contrast, it has been demonstrated that Sp1 does not physically interact with CBP/p300 (50, 51), although they co-precipitate in the same complexes (52). Requirement of CBP/p300 for transcriptional synergism between Sp1 and other transcription activators has been also demonstrated (40). Therefore, it is possible that HIF-1 and Sp1/Sp3 might coexist in the same cofactor complex to synergistically activate transcription of the RORα4 promoter. Alternatively, Sp1/Sp3 might interact directly with HIF-1. Although in the present study we detected no direct, physical interaction between HIF-1 and Sp1, it is possible that some other factor stabilizing their interaction might be involved in the hypoxia-induced activation of the RORα4 promoter. It has recently been shown that a direct interaction between these two transactivators could be further stabilized by Smad3, which interacts both with Sp1 and HIF-1 (53). The existence of the GGGAGG sequence that is the constituent of the hypoxia inducible region of the RORα4 promoter is also of interest. This sequence element has previously been identified as a binding site for ZBP-89, a Krüppel-type zinc finger transcription factor. ZBP-89 binds efficiently to this element and activates or represses transcription, depending on the promoters of the target genes (50, 54–57). Moreover, ZBP-89 has been shown to interact both with Sp1 and CBP/p300 (50). Although the present study showed that the major nuclear proteins bound with the GGGAGG sequence are Sp1/Sp3, a few protein complexes were observed that bound to the GA-box oligonucleotide but were not recognized by the antibodies for Sp1/Sp3 (see Fig. 4B). ZBP-89 might play a role in the collaboration of HIF-1 with Sp1 to activate the RORα4 promoter under hypoxia. Whether this is indeed the case requires further study.

To understand the possible roles of RORα in cellular adaptation to hypoxia, it is essential to identify its target genes.
Although there are several ROA-responsive element-containing genes that are expressed in the liver (7, 58–60), little is known about their association with hypoxia. As described above, several lines of evidence show that ROA has an anti-inflammatory effect. Lipopolysaccharide-activated macrophages in the 5g mice overproduce interleukin-1β and tumor necrosis factor α (30), suggesting that ROAs negatively regulate expression of such proinflammatory cytokines to set in motion a mechanism to limit inflammation. Interestingly, ROA has been suggested to interfere with the NF-κB signaling cascade by reducing its nuclear translocation (61). NF-κB plays a central role in a variety of cellular processes by regulating the transcription of a number of genes, including interleukin 1β, IL-6, tumor necrosis factor α, and cyclooxygenase-2 (62, 63). Recently, several stimuli other than oxygen tension have been shown to modulate HIF-1 expression and its consequent function. For example, interleukin 1β and tumor necrosis factor α have been demonstrated to stimulate the DNA binding activity of HIF-1 and have also been demonstrated to increase expression of ROA mRNA in endothelium and vascular muscle (19). The results obtained in the present study might support the possibility that the interleukin 1β tumor necrosis factor α-induced increase of ROA expression results from the activation of HIF-1. If this is the case, ROAs would be involved in a regulatory loop of proinflammatory cytokine production and would thereby play a protective role against inflammation and tissue injury associated with hypoxia. More studies are required to identify whether and how ROAs modulate the NF-κB signaling cascade and consequently regulates transcription of the proinflammatory cytokine genes. Identification of genes whose expressions correlate with the increased expression of ROA under hypoxia will help to define its function in the liver and in other tissues as well. Experiments along these lines are in progress.

REFERENCES

1. Mangeos, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1999) Cell 93, 835–839

2. Burris, T. P., and McCabe, E. R. B. (2001) Nuclear Receptor and Disease, Genetic Disease, Academic Press, Inc., New York

3. Jetten, A. M., Kurebayashi, S., and Ueda, E. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 205–209

4. Hamilton, B. A., Frankel, W. N., Korreblock, A. W., Hawkins, T. L., FitzHugh, J., and Bunn, H. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12969–12974

5. Mammonea, A., Seguret-Mace, S., Esposito, B., Chaniale, C., Bouly, M., Delhaye-Bouchaud, N., Kopmels, B., Wollman, E. E., Guastavino, J. M., Delhaye-Bouchaud, N., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1996) Nature 379, 736–739

6. Kussmaul, W., Lander, E. S., and Rudkin, B. B. (1999) J. Biol. Chem. 274, 277, 1371–1376

7. Billon, N., Carlisi, D., Datto, M. B., van Grunsven, L. A., Watt, A., Wang, X. F., and Fujii-Kuriyama, Y. (1999) Mol. Cell. Biol. 19, 2189–2197

8. Son, A. M., Beaufour, P. A., Robinson, K. M., Olinger, J. D., Arvisonis, D., Scheek, S., Szwarc, J., Kadonaga, J. T., and Tjian, R. (1996) Science 272, 420–420

9. Look, D. C., Pelletier, M. R., Timdrow, R. M., Roswit, W. M., and Holtzman, M. J. (1995) J. Biol. Chem. 270, 20626–20637

10. Mammonea, A., Seguret-Mace, S., Esposito, B., Chaniale, C., Bouly, M., Delhaye-Bouchaud, N., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12969–12973

11. Ebert, B. L., and Bunn, H. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5404–5408

12. Son, A. M., Beaufour, P. A., Robinson, K. M., Olinger, J. D., Arvisonis, D., Scheek, S., Szwarc, J., Kadonaga, J. T., and Tjian, R. (1996) Science 272, 420–420

13. Mammonea, A., Seguret-Mace, S., Esposito, B., Chaniale, C., Bouly, M., Delhaye-Bouchaud, N., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12969–12973

14. Ebert, B. L., and Bunn, H. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5404–5408

15. Mammonea, A., Seguret-Mace, S., Esposito, B., Chaniale, C., Bouly, M., Delhaye-Bouchaud, N., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12969–12973

16. Ebert, B. L., and Bunn, H. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5404–5408
Hypoxia-induced Activation of the Retinoic Acid Receptor-related Orphan Receptor α4 Gene by an Interaction between Hypoxia-inducible Factor-1 and Sp1
Naoki Miki, Megumi Ikuta and Takashi Matsui

J. Biol. Chem. 2004, 279:15025-15031.
doi: 10.1074/jbc.M313186200 originally published online January 23, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M313186200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 62 references, 38 of which can be accessed free at http://www.jbc.org/content/279/15/15025.full.html#ref-list-1