BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **223,** 54–59 (1996)
ARTICLE NO. 0845

Nucleotide Sequence, Chromosomal Assignment and mRNA Expression of Mouse Hypoxia-Inducible Factor-1α[1]

Roland H. Wenger,*[,2] Andreas Rolfs,* Hugo H. Marti,* Jean-Louis Guénet,† and Max Gassmann*

**Physiologisches Institut, Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland; †Unité de Génétique des Mammifères, Institut Pasteur, F-75724 Paris, France*

Received April 24, 1996

The heterodimeric hypoxia-inducible transcription factor HIF-1 is involved in the oxygen-regulated transcription of several genes including erythropoietin. Cloning and sequencing of the α-subunit of mouse HIF-1 cDNA revealed a 90% overall homology to human HIF-1α but lack of any similarity in the 5′ untranslated region and translational start site. Mouse HIF-1α is encoded by an evolutionary conserved single-copy gene located on chromosome 12. We found a widespread constitutive expression of mouse HIf-1α mRNA which was particularly high in lung and kidney. Despite a strong erythropoietin induction, HIF-1α mRNA concentrations were not upregulated in hypoxic mouse tissues.

The hypoxia-inducible factor-1 (HIF-1) has originally been defined by its capability of mediating hypoxic induction of erythropoietin (EPO) gene transcription (1). Subsequently, HIF-1 has also been reported to be implicated in the oxygen-dependent expression of several genes encoding glycolytic enzymes (2–4), the vascular endothelial growth factor (5,6), the VL30 retrotransposon (7), the inducible nitric oxide synthase (8), and the glucose transporter-1 (9). These findings, together with the notion that reporter gene constructs containing HIF-1 binding sites and DNA binding of HIF-1 are both hypoxically induced in several different cell lines (10–12), suggest that HIF-1 expression is widespread. A direct molecular demonstration of this assumption, however, is missing so far. Biochemical purification of human HIF-1 revealed a complex composed of two subunits tentatively termed HIF-1α and HIF-1β (13). Molecular cloning revealed that both subunits contain a basic-helix-loop-helix (bHLH) domain followed by a region of homology termed PAS (which stands for Per-AhR/ARNT-Sim, the prototype members of this family) (14). While HIF-1α is a new member of the bHLH-PAS family, HIF-1β is identical to the aryl hydrocarbon receptor (AhR) nuclear translocater (ARNT). Thus, ARNT dimerizes either with AhR to activate genes involved in the detoxification of xenobiotics such as dioxin, or with HIF-1α to induce oxygen-regulated gene expression. Human HIF-1α mRNA and protein expression have been shown to be induced by hypoxia in Hep3B hepatoma cells *in vitro* (14), but the *in vivo* expression profile is unknown. Here, we report on the molecular characterization of mouse HIF-1α mRNA, as well as on its expression in normoxic and hypoxic mouse tissues. Furthermore, we present evidence that HIF-1α is encoded by an evolutionary conserved single-copy gene (*Hif1a*) and identify the mouse chromosome bearing the *Hif1a* locus.

MATERIALS AND METHODS

Materials. Cell culture reagents and terminal deoxynucleotidyl transferase were obtained from LifeTechnologies (Basel, Switzerland), oligonucleotides from Microsynth (Balgach, Switzerland), BiodyneA nylon membranes from Pall (Winiger, Wohlen, Switzerland), Stratascript reverse transcriptase and pBluescript from Stratagene (Zürich, Switzerland), SuperTaq

[1] The nucleotide sequence reported in this paper has been deposited in the EMBL/GenBank/DDBJ databases (accession no. X95580).

[2] Corresponding author. Fax: +41 1 364 05 64. E-mail: labbauer@physiol.unizh.ch.

0006-291X/96 $18.00

DNA polymerase from Stehelin (Basel, Switzerland), restriction enzymes from MBI Fermentas (Mächler, Basel, Switzerland) and a T7 polymerase-based sequencing kit from Pharmacia (Dübendorf, Switzerland).

Cell culture and mRNA isolation. The mouse hepatoma cell line Hepa1c1c7 (15) was cultured in Dulbecco's modified Eagle's medium (high glucose) supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 × minimal essential medium non-essential amino acids, 2 mM L-glutamine and 1 mM Na-pyruvate in a humidified atmosphere containing 5% CO_2 at 37°C. Total RNA was isolated from subconfluent Hepa1c1c7 cells as described by Chomczynski and Sacchi (16).

Mouse HIF-1α cDNA sequencing. The core cDNA sequence of mouse HIF-1α was amplified by RT-PCR using the primers mHIF5.1 (5′-TGAGGCTCACCATCAGTTAT-3′) and mHIF3.1 (5′-CAGTATGCTCAAAATGTCTT-3′). The resulting 3.2 kb PCR fragment was subcloned into pBluescript and sequenced on both strands using an automatic DNA sequencer (ALF, Pharmacia). The flanking regions were obtained by 5′ and 3′ RACE as described previously (17). Briefly, the RT reaction for 5′ RACE was performed using the mouse HIF-1α primer HIF.RT (5′-AGCATTTCTCTCAT-3′) and Stratascript reverse transcriptase. Following the tailing reaction with terminal deoxynucleotidyl transferase in the presence of 0.1 mM dATP, the 5′ cDNA end was amplified by PCR using 0.2 μM $(dT)_{17}$adapter (5′-GACTCGAG-TCGACATCGA$(T)_{17}$-3′), 1 μM adapter (5′-GACTCGAGTCGACATCG-3′) and 1 μM HIF.AMP primer (5′-CCTCATGGTCACATGGA-3′). The RT reaction for 3′ RACE was performed with the $(dT)_{17}$adapter as primer, and the 3′ cDNA was amplified by PCR using the forward primer hHIF1a2134 (5′-TGAGGAAGAACTAAATCCAAAGA-3′) and the adapter (see above) as reverse primer. 5′ and 3′ RACE products were subcloned into pBluescript and sequenced with T7 polymerase according to the manufacturer's instructions (Pharmacia).

Southern blot analysis. Genomic DNA was isolated and analyzed from various tissues of different species using standard techniques (18). For Southern blot analysis, 10 μg DNA was digested with *Eco*RI and electrophoresed through 0.7% agarose gels. The DNA was transferred to BiodyneA membranes and crosslinked by UV irradiation (Stratalinker, Stratagene). Hybridization was performed in 6 × SSC, 10 × Denhardt's, 0.1% SDS, 1.1 mM $Na_4P_2O_7$, 17 mM Na_2HPO_4/NaH_2PO_4 (pH 7.7) and 200 μg/ml sonicated salmon sperm DNA at 62°C for 15 h. The blots were washed to a final stringency of 50°C in 0.2 × SSC, 0.1% SDS and radioactive signals were detected by autoradiography. A 0.5 kb fragment from the 5′ end of the human HIF-1α cDNA (kind gift of G.L. Semenza), labeled to a specific radioactivity of 1×10^9 dpm/μg by random-primed DNA labeling (18) was used as hybridization probe.

Chromosomal assignment. The genetic assignment of the *Hif1a* locus to a mouse chromosome was achieved by scoring the pattern of a restriction fragment length polymorphism (RFLP) on Southern blots made from highly informative DNA samples, prepared from a set of informative consomic strains having the C57BL/6 laboratory inbred strain (*Mus musculus*) and the STF/Pas inbred strain (*Mus spretus*) parental strains. Consomic strains are inbred strains where a complete chromosome of a given strain has been substituted for the homologous chromosome of another strain or species. DNA samples prepared from such a resource allow the very rapid chromosomal assignment of a cloned DNA sequence (J.-L. Guénet, unpublished data).

Northern blot analysis of normoxic and hypoxic mouse tissues. Hypoxic stimulation was achieved by exposing C57BL/6 mice to 0.1% carbon monoxide for 4 h as described (19). Total RNA was isolated from several organs and analyzed by Northern blotting using as probe a ^{32}P-labeled 2.2 kb cDNA fragment covering the coding region of mouse HIF-1α, as well as the control probes β-actin and 28S as described previously (20).

RESULTS AND DISCUSSION

Mouse HIF-1α cDNA Sequence

Using RNA isolated from the mouse hepatoma cell line Hepa1c1c7 (15), the core region of mouse HIF-1α was amplified by RT-PCR and sequenced. The mouse PCR primers were designed based on preliminary sequence data of PCR fragments obtained with primers derived from the human HIF-1α sequence (14). The 5′ and 3′ ends were obtained by the 5′ and 3′ RACE technique (17). A total of 8 independent 5′ RACE and 2 independent 3′ RACE products were sequenced and merged to the core sequence resulting in the full-length mouse HIF-1α cDNA shown in Fig. 1. Mouse and human HIF-1α share 90% homology on both the nucleotide and amino acid level including the bHLH and PAS domains. However, the most intriguing difference was found in the 5′ untranslated region (UTR) including the translational start site (Fig. 2a). Mouse HIF-1α lacks the first 12 amino acids present in human HIF-1α and the 125 bp 5′ UTR shares no homology to the 28 bp 5′ UTR reported for human HIF-1α (14). The mouse sequence was similar in all of the 8 sequenced 5′ RACE products, differing only in the length of the 5′ extensions. The 2 longest 5′ RACE products were used to tentatively designate the cap site of the HIF-1α mRNA. A more defined cap site requires the knowledge of the genomic sequence and mapping with complementary

```
cttcccccgtccacccatttctacccgccgggtggctcattcccctctcctgtaagcaaggagccagaatacatttttctctgccagttttctgggcaaact  100
                                    MetSerSerGluArgArgLysGluLysSerArgAspAlaAlaArgSerArgArgSerLysGluSerGluValPhe   25
gttaccgtgttagagcaattaaaggATGAGTTCTGAACGTCGAAAAGAAAAGTCTAGAGATGCAGCAAGATCTCGGCGAAGCAAAGAGTCTGAAGTTTTT  200
TyrGluLeuAlaHisGlnLeuProLeuProHisAsnValSerSerHisLeuAspLysAlaSerValMetArgLeuThrIleSerTyrLeuArgValArgL    59
TATGAGCTTGCTCATCAGTTGCCACTTCCCCACAATGTGAGCTCACATCTTGATAAAGCTTCTGTTATGAGGCTCACCATCAGTTATTTACGTGTGAGAA  300
ysLeuLeuAspAlaGlyGlyLeuAspSerGluAspGluMetLysAlaGlnMetAspCysPheTyrLeuLysAlaLeuAspGlyPheValMetValLeuTh   92
AACTTCTGGATGCCGGTGGTCTAGACAGTGAAGATGAGATGAAGGCACAGATGGACTGTTTTTATCTGAAAGCCCTAGATGGCTTTGTGATGGTGCTAAC  400
rAspAspGlyAspMetValTyrIleSerAspAsnValAsnLysTyrMetGlyLeuThrGlnPheGluLeuAlaGlyHisSerValPheAspPheThrHis   125
AGATGACGGCGACATGGTTTACATTTCTGATAACGTGAACAAATACATGGGGTTAACTCAGTTTGAACTAGCTGGACACAGTGTGTTTGATTTTACTCAT  500
ProCysAspHisGluGluMetArgGluMetLeuThrHisArgAsnGlyProValArgLysGlyLysGluLeuAsnThrGlnArgSerPhePheLeuArgM  159
CCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATGGCCCAGTGAGAAAAGGGAAAGAACTAAACACACAGCGGAGCTTTTTTCTCAGAA  600
etLysCysThrLeuThrSerArgGlyArgThrMetAsnIleLysSerAlaThrTrpLysValLeuHisCysThrGlyHisIleHisValTyrAspThrAs   192
TGAAGTGCACCCTAACAAGCCGGGGGAGGACGATGAACATCAAGTCAGCAACGTGGAAGGTGCTTCACTGCACGGGCCATATTCATGTCTATGATACCAA  700
nSerAsnGlnProGlnCysGlyTyrLysLysProProMetThrCysLeuValLeuIleCysGluProIleProHisProSerAsnIleGluIleProLeu   225
CAGTAACCAACCTCAGTGTGGGTACAAGAAACCACCCATGACGTGCTTGGTGCTGATTTGTGAACCCATTCCTCATCCGTCAAATATTGAAATTCCTTTA  800
AspSerLysThrPheLeuSerArgHisSerLeuAspMetLysPheSerTyrCysAspGluArgIleThrGluLeuMetGlyTyrGluProGluGluLeuL   259
GATAGCAAGACATTTCTCAGTCGACACAGCCTCGATATGAAATTTTCTTACTGTGATGAAAGAATTACTGAGTTGATGGGTTATGAGCCGGAAGAACTTT  900
euGlyArgSerIleTyrGluTyrTyrHisAlaLeuAspSerAspHisLeuThrLysThrHisHisAspMetPheThrLysGlyGlnValThrThrGlyGl   292
TGGGCCGCTCAATTTATGAATATTATCATGCTTTGGATTCTGATCATCTGACCAAAACTCACCATGATATGTTTACTAAAGGACAAGTCACCACAGGACA 1000
nTyrArgMetLeuAlaLysArgGlyGlyTyrValTrpValGluThrGlnAlaThrValIleTyrAsnThrLysAsnSerGlnProGlnCysIleValCys   325
GTACAGGATGCTTGCCAAAAGAGGTGGATATGTCTGGGTTGAAACTCAAGCAACTGTCATATATAATACGAAGAACTCCCAGCCACAGTGCATTGTGTGT 1100
ValAsnTyrValValSerGlyIleIleGlnHisAspLeuIlePheSerLeuGlnGlnThrGluSerValLeuLysProValGluSerSerAspMetLysM   359
GTGAATTATGTTGTAAGTGGTATTATTCAGCACGACTTGATTTTCTCCCTTCAACAAACAGAATCTGTGCTCAAACCAGTTGAATCTTCAGATATGAAGA 1200
etThrGlnLeuPheThrLysValGluSerGluAspThrSerCysLeuPheAspLysLeuLysLysGluProAspAlaLeuThrLeuLeuAlaProAlaAl   392
TGACTCAGCTGTTCACCAAAGTTGAATCAGAGGATACAAGCTGCCTTTTTGATAAGCTTAAGAAGGAGCCTGATGCTCTCACTCTGCTGGCTCCAGCTGC 1300
aGlyAspThrIleIleSerLeuAspPheGlySerAspAspThrGluThrGluAspGlnGlnLeuGluAspValProLeuTyrAsnAspValMetPhePro   425
CGGCGACACCATCATCTCTCTGGATTTTGGCAGCGATGACACAGAAACTGAAGATCAACAACTTGAAGATGTTCCATTATATAATGATGTAATGTTTCCC 1400
SerSerAsnGluLysLeuAsnIleAsnLeuAlaMetSerProLeuProSerSerGluThrProLysProLeuArgSerSerAlaAspProAlaLeuAsnG   459
TCTTCTAATGAAAAATTAAATATAAACCTGGCAATGTCTCCTTTACCTTCATCGGAAACTCCAAAGCCACTTCGAAGTAGCGCTGATCCTGCACTGAATC 1500
lnGluValAlaLeuLysLeuGluSerSerProGluSerGluGlyLeuSerPheThrMetProGlnIleGlnAspGlnProAlaSerProSerAspGlySe   492
AAGAGGTTGCATTAAAATTAGAATCAAGTCCAGAGTCACTGGGACTTTCTTTTACCATGCCCCAGATTCAAGATCAGCCAGCAAGTCCTTCTGATGGAAG 1600
rThrArgGlnSerSerProGluProAsnSerProSerGluTyrCysPheAspValAspSerAspMetValAsnValPheLysLeuGluLeuValGluLys   525
CACTAGACAAAGTTCACCTGAGCCTAACAGTCCCAGTGAATATTGCTTTGATGTGGATAGCGATATGGTCAATGTATTCAAGTTGGAACTGGTGGAAAAA 1700
LeuPheAlaGluAspThrGluAlaLysAsnProPheSerThrGlnAspThrAspLeuAspLeuGluMetLeuAlaProTyrIleProMetAspAspAspP   559
CTGTTTGCTGAAGACACAGAGGCAAAGAATCCATTTTCAACTCAGGACACTGATTTAGATTTGGAGATGCTGGCTCCCTATATCCCAATGGATGATGATT 1800
heGlnLeuArgSerPheAspGlnLeuSerProLeuGluSerAsnSerProSerProProSerMetSerThrValThrGlyPheGlnGlnThrGlnLeuGl   592
TCCAGTTACGTTCCTTTGATCAGTTGTCACCATTAGAGAGCAATTCTCCAAGCCCTCCAAGTATGAGCACAGTTACTGGGTTCCAGCAGACCCAGTTACA 1900
nLysProThrIleThrAlaThrAlaThrThrThrAlaThrThrAspGluSerLysThrGluThrLysAspAsnLysGluAspIleLysIleLeuIleAla   625
GAAACCTACCATCACTGCCACTGCCACCACAACTGCCACCACTGATGAATCAAAAACAGAGACGAAGGACAATAAAGAAGATATTAAAATACTGATTGCA 2000
SerProSerSerThrGlnValProGlnGluThrThrThrAlaLysAlaSerAlaTyrSerGlyThrHisSerSerArgThrAlaSerProAspArgAlaGlyL   659
TCTCCATCTTCTACCCAAGTACCTCAAGAAACGACCACTGCTAAGGCATCAGCATACAGTGGCACTCACAGTCGGACAGCCTCACCAGACAGAGCAGGAA 2100
ysArgValIleGluGlnThrAspLysAlaHisProArgSerLeuAsnLeuSerAlaThrLeuAsnGlnArgAsnThrValProGluGluGluLeuAsnPr   692
AGAGAGTCATAGAACAGACAGACAAAGCTCATCCAAGGAGCCTTAACCTGTCTGCCACTTTGAATCAAAGAAATACTGTTCCTGAGGAAGAATTAAACCC 2200
oLysThrIleAlaSerGlnAsnAlaGlnArgLysArgLysMetGluHisAspGlySerLeuPheGlnAlaAlaGlyIleGlyThrLeuLeuGlnGlnPro   725
AAAGACAATAGCTTCGCAGAATGCTCAGAGGAAGCGAAAAATGGAACATGATGGCTCCCTTTTTCAAGCAGCAGGAATTGGAACATTATTGCAGCAACCA 2300
GlyAspCysAlaProThrMetSerLeuSerTrpLysArgValLysGlyPheIleSerSerGluGlnAsnGlyThrGluGlnLysThrIleIleLeuIleP   759
GGCGACTGTGCACCTACTATGTCACTTTCCTGGAAACGAGTGAAAGGATTCATATCTAGTGAACAGAATGGAACGGAGCAAAAGACTATTATTTTAATAC 2400
roSerAspLeuAlaCysArgLeuLeuGlyGlnSerMetAspValSerGlyLeuProGlnLeuThrSerTyrAspCysGluValAsnAlaProIleGlnGl   792
CCTCCGATTTAGCATGCAGACTGCTGGGGCAGTCAATGGATGTGAGTGGATTACCACAGCTGACCAGTTACGATTGTGAAGTTAATGCTCCCATACAAGG 2500
ySerArgAsnLeuLeuGlnGlyGluGluLeuArgAlaLeuAspGlnValAsn                                                    810
CAGCAGAAACCTACTGCAGGGTGAAGAATTACTCAGAGCTTTGGATCAAGTTAACTGAgcgtttcctaatctcattccttttgattgttaatgtttttgt 2600
tcagttgttgttgtttgttgggtttttgtttctgttggttatttttggacactggtggctcagcagtctatttatatttttctatatctaattttagaagc 2700
ctggctacaatactgcacaaactcagatagtttagttttcatccccttttctacttaatttttcattaatgctcttttttaatatgttcttttaatgccagat 2800
cacagcacattcacagctcctcagcatttcaccattgcattgctgtagtgtcatttaaaatgcacctttttatttatttatttttggtgagggagtttgt 2900
cccttattgaattatttttaatgaaatgccaatataatttttttaagaaagcagtaaattctcatcatgatcataggcagttgaaaacttttactcattt 3000
ttttcatgttttacatgaaaataatgctttgttagcagtacatggtagccacaattgcacaatatattttcttttaaaaaaccagcagttactcatgcaat 3100
atattctgcatttataaaactagttttttaggaaatttttttttggcctatggaattgttaagcctggatcatgaagctgttgatcttataatgattcttaa 3200
actgtatggtttcttttatatgggtaaagccatttacatgatataaagaaatatgcttatatctggaaggtatgtggcatttatttggataaaattctcaa 3300
ttcagagaagttatctggtgtttcttgacttttaccaactcaaaacagcccctctgtagttgtggaagcttatgctaatattgtgtaattgattatgaaac 3400
ataaatgttctgcccaccctgttggtataaagacattttgagcatactgtaaacaaacaaacaaaaaatcatgctttgttagtaaaattgcctagtatgt 3500
tgatttgttgaaaatatgatgtttggttttatgcactttgtcgctattaacatccttttttcatatagatttcaataagtgagtaatttttagaagcatta 3600
ttttaggaatatagagttgtcatagtaaacatcttgtttttttctatgtatactgtataaatttttcgttcccttgctctttgtggttgggtctaacacta 3700
actgtactgttttgttatatcaaataaacatcttctgtggaccagg                                                        3746
```

FIG. 1. Nucleotide and deduced amino acid sequence of mouse HIF-1α. Coding sequences are in capital letters and UTR's in small letters. The bHLH domain is in bold and the PAS (A) and (B) domains are in italics, AUUUA mRNA instability elements are underlined, and the polyadenylation signal is double underlined.

methods such as RNase protection. Another remarkable difference was found at the beginning of the 3′ UTR where the mouse sequence contains a 61 bp long GT-rich insertion not present in the human sequence (Fig. 2b). The rest of the 3′ UTR, however, is again well conserved including the polyadenylation signal and the polyadenylation site. The mouse HIF-1α 3′ UTR contains 7 AUUUA mRNA instability elements (20) compared to the human sequence which contains 8 such elements (14). Five of these elements are conserved between mouse and human HIF-1α.

FIG. 2. Sequence comparison of the regions with greatest variability between mouse and human (13) HIF-1α cDNA. (a) 5′ UTR and translational start sites are different in the two species. The lengths of the UTR's are indicated by a number on top of the arrows. (b) Mouse HIF-1α contains a GT-rich insertion 3′ to the stop codon which is not present in the human cDNA sequence. The positions of the stop codons are indicated by arrows and the respective number.

HIF-1α Is Encoded by an Evolutionary Conserved Single-Copy Gene

Southern blot analysis of genomic mouse DNA digested with *Eco*RI yielded a single band when probed with a 0.5 kb human HIF-1α 5′ cDNA probe (Fig. 3). Digestion with 5 other 6 bp-cutting restriction enzymes also revealed only a single band (data not shown) suggesting that mouse HIF-1α is encoded by a single-copy gene. As demonstrated by a "zoo-blot" analysis, the *Hif1a* gene is likely to be well-conserved among mammalian and avian species (Fig. 3A). Distinct bands were also detected in drosophila and yeast when the same hybridization conditions were applied that revealed a single *Hif1a* signal in mouse (Fig. 3B). Of note, a drosophila cDNA has recently been cloned (accession no. U43090) which is 59% identical to mouse HIF-1α over a stretch of 1155 nucleotides, suggesting that it might represent the drosophila HIF-1α homologue.

Assignment of the Hif1a Locus to Mouse Chromosome 12

The restriction endonuclease *Eco*RI generated a clear RFLP when used for the digestion of DNA samples in Southern blot analysis of the parental strains of our consomic samples. With this enzyme, the HIF-1α probe hybridized to a 8.9 kb band with DNA samples of *Mus musculus* (C57BL/6) and a 7.5 kb diagnostic band with *Mus spretus* (STF/Pas) DNA samples. The analysis of the RFLP pattern among a set of 18 DNA samples indicated that the *Hif1a* locus maps unambiguously to mouse chromosome 12. Whereas the *Ahr* gene resides also on chromosome 12 (22), *Arnt* and *Sim* are located on mouse chromosomes 3 and 16, respectively (23,24), indicating that the bHLH-PAS family of transcription factors is not clustered. A more detailed mapping will be necessary to determine the relative positions of *Hif1a* and *Ahr* on chromosome 12.

HIF-1α mRNA Is Constitutively Expressed in Normoxic and Hypoxic Mouse Tissues

Human HIF-1α mRNA, protein and DNA-binding have been reported to be at very low levels in normoxic Hep3B and HeLa cells, and upregulated following exposure to hypoxia for 4 h *in vitro* (14). To examine the constitutive and hypoxia-inducibility expression of mouse HIF-1α mRNA *in*

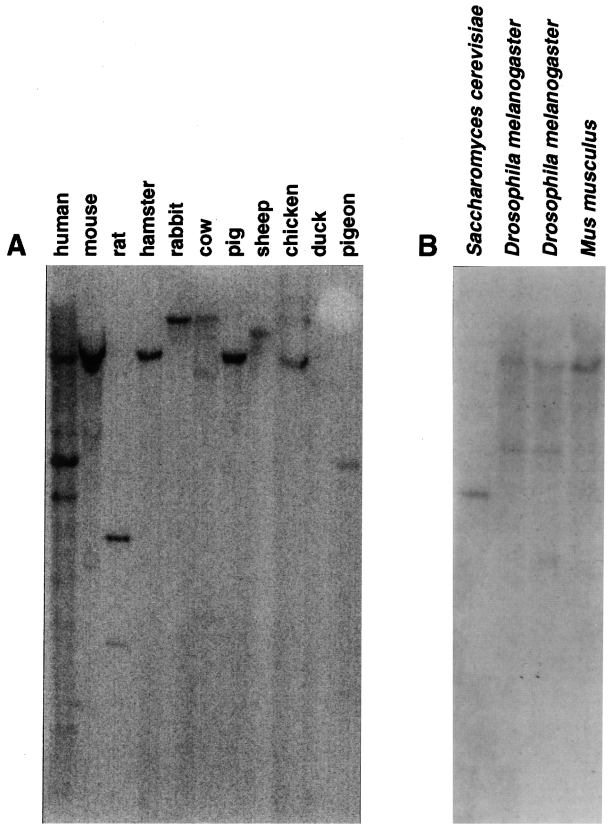

FIG. 3. Southern blot analysis of genomic DNA derived from different species. Genomic DNA digested with *Eco*RI was probed with a human HIF-1α cDNA fragment covering the bHLH and PAS A domains. Hybridization and washing conditions were identical in (A) and (B). Two different Drosophila samples were loaded.

vivo, groups of 3 adult mice were either exposed to normal air (Fig. 4, mouse no. 1 to 3) or to 0.1% carbon monoxide (Fig. 4, mouse no. 4 to 6) resulting in "functional anaemia" (25) and thus tissue hypoxia. Like we reported previously, hypoxic induction of gene expression was confirmed in the same mice by a 40-fold induction of EPO serum protein levels and a more than 100-fold induction of EPO mRNA levels in kidney and liver (19), as measured in the same mRNA samples shown in

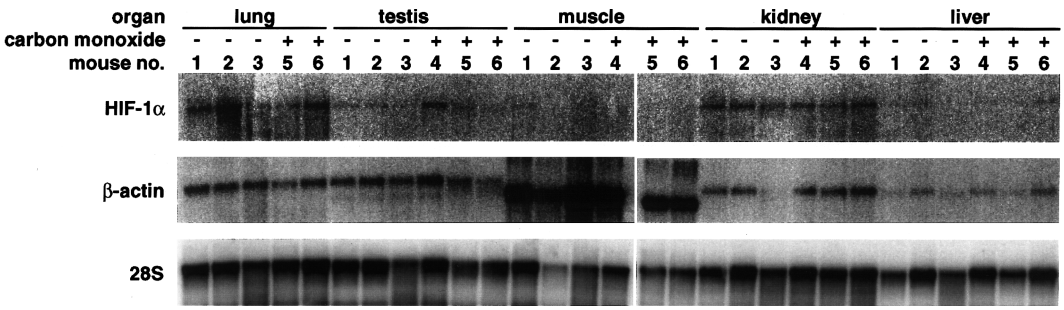

FIG. 4. Northern blot analysis of normoxic and hypoxic mouse tissues. Total RNA derived from different organs of either untreated mice (no. 1–3) or mice that have been exposed to 0.1% carbon monoxide for 4 h (no. 4–6), resulting in tissue hypoxia, was probed with a 2.2 kb mouse HIF-1α cDNA fragment containing the coding region. β-actin and ribosomal 28S RNA hybridization probes were subsequently applied to the same blot to correct for loading and blotting differences.

Fig. 4. Total RNA isolated from lung, testis, skeletal muscle, kidney and liver was analyzed by Northern blotting and hybridization to a 2.2 kb mouse HIF-1α cDNA probe encompassing the coding region (Fig. 4). In the normoxic control mice, constitutive HIF-1α mRNA expression was most prominent in lung and kidney and less detectable in muscle and liver. However, following prolonged exposure, a HIF-1α signal was found in all organs tested, suggesting that HIF-1α is ubiquitously expressed. Despite some variation among the individual animals, HIF-1α mRNA was not significantly induced in any of the hypoxic tissues examined (Fig. 4). Since we were also unable to detect hypoxic HIF-1α mRNA upregulation in several different cell lines, again under conditions where several oxygen-regulated genes were induced (data not shown), we conclude that either the kinetics of HIF-1α mRNA expression does not parallel the expression of HIF-1α-dependent genes, or translational mechanisms and/or post-translational modifications regulate HIF-1-dependent hypoxic induction of gene expression.

ACKNOWLEDGMENTS

We thank M. Szatanik for expert technical assistance; R. Städeli, D. Zimmermann and H. Hilfiker for technical advice; G. L. Semenza for providing the human HIF-1α cDNA probe; H.-M. Fischer for providing genomic DNA samples; C. Gasser for the artwork; and J. Silke and C. Bauer for critically reading the manuscript. This work was supported by the Swiss National Science Foundation (Grant 31-36369.92) to M.G. and the Olga-Mayenfisch-Stiftung to R.H.W. The development of the consomic DNA resource was made possible by the help of a grant from the GREG-GIP to J.-L.G. R.H.W. is a recipient of the "Sondermassnahmen des Bundes zur Förderung des akademischen Nachwuchses."

REFERENCES

1. Semenza, G. L., and Wang, G. L. (1992) *Mol. Cell. Biol.* **12,** 5447–5454.
2. Firth, J. D., Ebert, B. L., Pugh, C. W., and Ratcliffe, P. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6496–6500.
3. Firth, J. D., Ebert, B. L., and Ratcliffe, P. J. (1995) *J. Biol. Chem.* **270,** 21021–21027.
4. Semenza, G. L., Roth, P. H., Fang, H.-M., and Wang, G. L. (1994) *J. Biol. Chem.* **269,** 23757–23763.
5. Liu, Y., Cox, S. R., Morita, T., and Kourembanas, S. (1995) *Circ. Res.* **77,** 638–643.
6. Levy, A. P., Levy, N. S., Wegner, S., and Goldberg, M. A. (1995) *J. Biol. Chem.* **270,** 13333–13340.
7. Estes, S. D., Stoler, D. L., and Anderson, G. R. (1995) *J. Virology* **69,** 6335–6341.
8. Melillo, G., Musso, T., Sica, A., Taylor, L. S., Cox, G. W., and Varesio, L. (1995) *J. Exp. Med.* **182,** 1683–1693.
9. Ebert, B. L., Firth, J. D., and Ratcliffe, P. J. (1995) *J. Biol. Chem.* **270,** 29083–29089.
10. Wang, G. L., and Semenza, G. L. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4304–4308.
11. Maxwell, P. H., Pugh, C. W., and Ratcliffe, P. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2423–2427.
12. Kvietikova, I., Wenger, R. H., Marti, H. H., and Gassmann, M. (1995) *Nucleic Acids Res.* **23,** 4542–4550.
13. Wang, G. L., and Semenza, G. L. (1995) *J. Biol. Chem.* **270,** 1230–1237.
14. Wang, G. L., Jiang, B.-H., Rue, E. A., and Semenza, G. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5510–5514.
15. Hankinson, O. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 373–376.
16. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
17. Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J. (1990) PCR Protocols, Academic Press, San Diego, CA.
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY.
19. Marti, H. H., Wenger, R. H., Rivas, L. A., Straumann, U., Digicaylioglu, M., Henn, V., Yonekawa, Y., Bauer, C., and Gassmann, M. (1996) *Eur. J. Neurosci.* **8,** 666–676.
20. Wenger, R. H., Rolfs, A., Marti, H. H., Bauer, C., and Gassmann, M. (1995) *J. Biol. Chem.* **270,** 27865–27870.
21. Lagnado, C. A., Brown, C. Y., and Goodall, G. J. (1994) *Mol. Cell. Biol.* **14,** 7984–7995.
22. Schmidt, J. V., Carver, L. A., and Bradfield, C. A. (1993) *J. Biol. Chem.* **268,** 22203–22209.
23. Johnson, B., Brooks, B. A., Heinzmann, C., Diep, A., Mohandas, T., Sparkes, R. S., Reyes, H., Hoffman, E., Lange, E., Gatti, R. A., Xia, Y.-R., Lusis, A. J., and Hankinson, O. (1993) *Genomics* **17,** 592–598.
24. Ema, M., Suzuki, M., Morita, M., Hirose, K., Sogawa, K., Matsuda, Y., Gotoh, O., Saijoh, Y., Fujii, H., Hamada, H., and Fujii-Kuriyama, Y. (1996) *Biochem. Biophys. Res. Com.* **218,** 588–594.
25. Eckardt, K.-U., Ratcliffe, P. J., Tan, C. C., Bauer, C., and Kurtz, A. (1992) *J. Clin. Invest.* **89,** 753–760.