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Abstract

The human hypoxia-inducible transcription factor HIF-1 is a critical regulator of cellular and systemic responses to low oxygen levels. When oxygen levels are high, the HIF-1α subunit is hydroxylated and is targeted for degradation by the von Hippel-Lindau tumor suppressor protein (VHL). This regulatory pathway is evolutionarily conserved, and the *Caenorhabditis elegans hif-1* and *vhl-1* genes encode homologs of the HIF-1α subunit and VHL. To understand and describe more fully the molecular basis for hypoxia response in this important genetic model system, we compared hypoxia-induced changes in mRNA expression in wild-type, *hif-1*-deficient, and *vhl-1*-deficient *C. elegans* using whole genome microarrays. These studies identified 110 hypoxia-regulated gene expression changes, 63 of which require *hif-1* function. Mutation of *vhl-1* abrogates most *hif-1*-dependent changes in mRNA expression. Genes regulated by *C. elegans hif-1* have predicted functions in signal transduction, metabolism, transport, and extracellular matrix remodeling. We examined the *in vivo* requirement for 16 HIF-1 target genes and discovered that the *phy-2* prolyl 4-hydroxylase α subunit is critical for survival in hypoxic conditions. Some HIF-1 target genes negatively regulate formation of stress-resistant dauer larvae. The microarray data presented herein also provide clear evidence for an HIF-1-independent pathway for hypoxia response, and this pathway regulates the expression of multiple heat shock proteins and several transcription factors.

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Roles of the HIF-1 Hypoxia-inducible Factor during Hypoxia Response in *Caenorhabditis elegans**[S]

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The human hypoxia-inducible transcription factor HIF-1 is a critical regulator of cellular and systemic responses to low oxygen levels. When oxygen levels are high, the HIF-1α subunit is hydroxylated and is targeted for degradation by the von Hippel-Lindau tumor suppressor protein (VHL). This regulatory pathway is evolutionarily conserved, and the *Caenorhabditis elegans hif-1* and *vhl-1* genes encode homologs of the HIF-1α subunit and VHL. To understand and describe more fully the molecular basis for hypoxia response in this important genetic model system, we compared hypoxia-induced changes in mRNA expression in wild-type, *hif-1*-deficient, and *vhl-1*-deficient *C. elegans* using whole genome microarrays. These studies identified 110 hypoxia-regulated gene expression changes, 63 of which require *hif-1* function. Mutation of *vhl-1* abrogates most *hif-1*-dependent changes in mRNA expression. Genes regulated by *C. elegans hif-1* have predicted functions in signal transduction, metabolism, transport, and extracellular matrix remodeling. We examined the *in vivo* requirement for 16 HIF-1 target genes and discovered that the *phy-2* prolyl 4-hydroxylase α subunit is critical for survival in hypoxic conditions. Some HIF-1 target genes negatively regulate formation of stress-resistant dauer larvae. The microarray data presented herein also provide clear evidence for an HIF-1-independent pathway for hypoxia response, and this pathway regulates the expression of multiple heat shock proteins and several transcription factors.

During development, homeostasis, or disease states, cellular oxygen levels are often insufficient to meet physiological demands, and this condition is termed hypoxia. Mammalian cells respond to hypoxia by implementing changes in gene expression to increase anaerobic energy production, protect cells from stress, regulate cell survival, and increase local angiogenesis. The requisite changes in gene expression are largely controlled by the hypoxia-inducible factor 1 (HIF-1)[1] transcription factor (1, 2).

HIF-1 is a heterodimeric DNA-binding complex, and both subunits are members of the family of transcription factors containing basic-helix-loop-helix and Per-ARNT-Sim domains. The HIF-1β subunit is also termed ARNT (aryl hydrocarbon receptor nuclear translocator). Although ARNT can dimerize with other transcription factors, HIF-1α is apparently dedicated to a hypoxia response (3–5). When oxygen levels are high, specific proline residues of HIF-1α are hydroxylated by oxygen-dependent enzymes belonging to the EGL-9/PH superfamily of 2-oxoglutarate-dependent dioxygenases (6, 7). Proline hydroxylation in the conserved L*XX*LAP motif in HIF-1α increases its affinity for the von Hippel-Lindau tumor suppressor protein (VHL), which is part of an E3 ubiquitin-ligase complex that targets proteins for proteasomal degradation. Thus, when VHL is disabled by mutation, HIF-1 is expressed at constitutively high levels (8).

Cells utilize multiple strategies to regulate HIF-1α activity. HIF-1α is modified post-translationally by hydroxylation, phosphorylation, and acetylation (7, 9, 10). Oxygen-dependent hydroxylation of the HIF-1α C terminus inhibits binding to the coactivator CBP/p300 (11, 12). Growth factor stimulation may modulate HIF-1α transcriptional activity via small GTPases and mitogen-activated protein kinase cascades (13, 14). The availability of interacting proteins, such as transcriptional coactivators, also influences HIF-1 function. Important questions remain. What other oxygen-sensing molecules control cellular hypoxia response? What are the most important VHL-independent mechanisms for regulating HIF-1 activity? What fraction of the transcriptional response to hypoxia is controlled by HIF-1?

Caenorhabditis elegans has recently proven to be an important model system for studying hypoxia response. The *C. elegans* homolog of the HIF-1α subunit is *hif-1*, and the HIF-1/EGL-9/VHL pathway is evolutionarily conserved (6, 15). *C. elegans* carrying deletions in the *hif-1* gene are apparently healthy in standard culture conditions, but they exhibit high levels of lethality in 0.5 or 1% oxygen (15, 16). To identify genes regulated by HIF-1 during response to hypoxia and to determine what fraction of hypoxia-induced gene expression changes is dependent upon *hif-1*, we conducted genomewide studies of hypoxia-induced changes in mRNA expression in wild-type, *hif-1*-deficient, and *vhl-1*-deficient *C. elegans*. These studies demonstrate that *C. elegans hif-1* regulates the majority of early transcriptional responses to hypoxia and provide clear evidence for HIF-1-independent pathways for adaptation to oxygen deprivation.

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[S] The on-line version of this article (available at http://www.jbc.org) contains Tables S1–S3.

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[1] The abbreviations used are: HIF-1, hypoxia-inducible factor 1; CBP, cAMP-response element-binding protein-binding protein; E3, ubiquitin-protein isopeptide ligase; eEF-2, elongation factor 2; GFP, green fluorescent protein; HRE, hypoxia response element; PHD, prolyl hydroxylase; RNAi, RNA interference; VHL, von Hippel-Lindau.

EXPERIMENTAL PROCEDURES

C. elegans Culture, Microarray Experiments, and Data Analysis—*C. elegans* were cultured using standard procedures on NGM plates with an *Escherichia coli* food source (17). For hypoxia treatments, plates containing synchronized third stage larvae were placed either in room air (normoxia control) or a sealed plexiglass chamber with constant gas flow. Compressed air and pure nitrogen were mixed to achieve the appropriate oxygen concentration, as assayed by an oxygen sensor (15). We were most interested in the earliest *hif-1*-dependent and *hif-1*-independent responses to hypoxia. Therefore, we assayed mRNA expression at the earliest time point at which the F22B5.4 transcript was consistently induced (4 h at 21 °C in 0.1% oxygen). At the time that this study was conducted, F22B5.4 was the only *C. elegans* transcript shown to be induced by hypoxia. The F22B5.4 putative 5′-regulatory sequences contain multiple elements similar to the mammalian hypoxia response element (HRE), the DNA binding site for the HIF-1 transcriptional complex, and F22B5.4 is expressed at high levels in *egl-9* mutants (6). These data suggested that F22B5.4 might be a direct target for *C. elegans* HIF-1. For the microarray experiments, three strains were assayed: wild-type N2, *hif-1* (*ia04*), and *vhl-1* (*ok161*). Worms were incubated for 4 h in 21% oxygen or 0.1% oxygen at 21 °C. Animals were quickly harvested in ice-cold M9 buffer, and poly(A) RNA was isolated using established procedures. No more than 3 min elapsed between the removal of plates from the hypoxic chamber and the addition of TRIzol.

The microarray hybridizations were performed by Min Jiang in the laboratory of Stuart K. Kim at Stanford University (18). cDNA from animals incubated in 21% O_2 (normoxia) was labeled with Cy5, and cDNA from hypoxia-treated worms was labeled with Cy3. There were 18 mRNA samples: (two oxygen concentrations × three genotypes × three independent experiments). Each hybridization compared expression in normoxia *versus* hypoxia for one genotype, and nine experiments were performed. The raw data and the normalized data are available at the Stanford Microarray Data base. The following criteria were used to filter the data: FLAG = 0; failed = 0; red and green fluorescence intensity at least 2-fold above background. We averaged the $\log_2$ values (hypoxia net intensity/normalized normoxia net intensity) from each experiment. Student's t test p values were determined with the averages for each strain.

To identify putative regulatory elements in *hif-1*-dependent genes, we used the motif-finding program at bioprospector.stanford.edu (BioProspector). We searched for DNA sequences that were overrepresented in the 18 genes most strongly induced by hypoxia in Table I relative to the 47 *hif-1*-independent genes. Sequences 200–2,000 bp upstream from each putative translational start were used for this analysis. Gene cluster analyses were performed using tools available at PlantGDB (19).

Viability Assays—To create DNA constructs for bacteria-mediated RNAi, cDNAs provided by Yuji Kohara were subcloned into the L4440 double-T7 vector (20). When cDNAs were not available, coding sequences were PCR amplified using primers designed by Valerie Reinke (21). The resulting constructs were transformed into *E. coli* strain HT115 (DE3) (20). RNAi analyses were performed as described previously (22). Additionally, embryonic and larval viability was assayed in the following mutant strains: *hif-1* (*ia04*), *cam-1* (*gm122*), *egl-9* (*sa307*), *fmo-12* (*ok299*), *fmo-14* (*ok294*), *inx-2* (*ok376*), *phy-2* (*ok177*), and *vab-1* (*e2*). Assays for viability at 21 or 0.5% O_2 at 21 °C were performed as described previously (16). Three independent experiments were conducted for each condition, and Student's t test was used to judge whether averaged values differed significantly.

RNA Blots—Total RNAs were isolated from synchronized L3 stage wild-type (Bristol strain N2) worms, *hif-1* (*ia04*) mutants, *vhl-1* (*ok161*) mutants, and *hif-1* (*ia04*) *vhl-1* (*ok161*) double mutants that had been incubated for 4 h in normoxia or hypoxia (0.5% O_2). The RNA samples were fractionated via agarose-formaldehyde gel electrophoresis and transferred to nylon membranes. DNA probes for RNA blot analyses were amplified using published primers (21). Hybridizations were performed using [α-^{32}P]dCTP-labeled probes, and signals were detected and quantified using a phosphorimager.

GFP Reporter Analysis—To generate the *phy-2*:GFP construct pSH01, DNA was PCR amplified using the primers Phy2PF2 (PstI) 5′-ggcgctgcagAGACTATAGTCTATAGCTGAAAACG and Phy2PR2 (BamHI) 5′-gcgggatccACTGCTCTCATTCTGAAAGACAAATC. The PCR product includes the *phy-2* sequence from −1715 bp to +11 bp relative to the translational start site (23). These regulatory sequences were cloned into GFP vector pPD95.75 (provided by A. Fire, Carnegie Institution of Washington). To create transgenic animals, the DNA was coinjected with the pRF-4 *rol-6* marker (24). Five independent transgenic lines were examined.

RESULTS

The primary goals of these studies were to identify hypoxia-induced gene expression changes in *C. elegans* and to determine which of these responses to hypoxia were regulated by *hif-1*. Toward these aims, we analyzed mRNA expression patterns in synchronized populations of wild-type worms that were cultured in standard laboratory conditions (normoxia) or in hypoxia. We also assayed mRNA from two mutant strains: (*a*) animals carrying the strong loss-of-function mutation in *hif-1* and (*b*) *C. elegans* that carry a deletion in *vhl-1* and express the HIF-1 protein at constitutively high levels. RNA was isolated from three independent experiments for each experimental condition (three genotypes; normoxia *versus* hypoxia). mRNAs were hybridized to whole genome microarrays that contained probes for 17,817 predicted genes (94% of the genome). To identify hypoxia-induced gene expression changes in wild-type worms, we used two criteria. First, we identified genes that exhibited a significant difference in mRNA levels in hypoxia *versus* normoxia as judged by Student's t test, p value <0.05. Second, we focused on genes that showed an average hypoxia-induced difference in mRNA expression greater than or equal to 2-fold. 110 genes met both of these criteria. To determine which of these gene expression changes were dependent upon *hif-1* function, we examined the data from *hif-1* (*ia04*) mutants. 63 of the 110 hypoxia-responsive genes were not significantly regulated by hypoxia in *hif-1* (*ia04*) mutants (p value $>$ 0.05), and they are listed in Table I. In this article, we will refer to this class of genes as "*hif-1*-dependent." The 47 genes listed in Table II were expressed at significantly higher or lower levels in hypoxia in *hif-1*-deficient animals (p value < 0.05; Table II), and we will refer to them as "*hif-1*-independent" genes. In most cases, hypoxia caused increased gene expression, but 6 of the *hif-1*-dependent genes and 3 of the *hif-1*-independent genes were down-regulated by hypoxia. A total of 654 genes met less stringent criteria for hypoxia-responsive genes (1.5-fold average difference between hypoxia and normoxia treatments; $p <$ 0.05), and they are listed in the supplemental data.

To validate further the microarray data and to compare directly the expression of certain genes in the presence or absence of *hif-1* and *vhl-1* function, we analyzed the expression of a subset of the hypoxia-induced genes on RNA blots. *C. elegans* hypoxia has been defined as oxygen concentrations between 0.1 and 2% (25, 26). To confirm that the gene expression changes detected by our microarray analyses were induced by another oxygen concentration in this range, we repeated the experiments using 0.5% oxygen. We tested eight of the *hif-1*-dependent genes. One of these genes, F57B9.1, was not expressed at levels detectable by RNA blot analysis. The other seven genes were (*a*) induced by hypoxia in wild-type animals; (*b*) expressed at lower levels in *hif-1*-defective mutants; (*c*) expressed at higher levels in animals lacking *vhl-1* function; and (*d*) expressed at low levels in double mutants, consistent with VHL-1 acting as a regulator of HIF-1 (Fig. 1*A*). Genes were chosen for RNA blot analysis based on signal intensity, level of differential expression, and, in some cases, predicted function. The seven *hif-1*-dependent genes tested (and their predicted functions) were *fmo-12* (flavin-containing monooxygenase), F22B5.4 (unknown function), W07A12.7 (acyltransferase), *phy-2* (prolyl 4-hydroxylase α subunit), *nhr-57* (nuclear hormone receptor), *egl-9* (2-oxoglutarate-dependent oxygenase), and K10H10.2 (cysteine synthase). We assayed four of the *hif-1*-independent genes listed in Table II by RNA blots. They were F26A3.4 (phosphatase), C12C8.1 (Hsp70), F44E5.5 (Hsp70), and R166.5 (serine/threonine kinase). All four were induced by hypoxia in wild-type animals and in mutants lacking *hif-1* or *vhl-1* function (Fig. 1*B*).

TABLE I
63 genes regulated by hypoxia in a hif-1-dependent manner

Gene	Description	Wild type H/N ratio[a] (*p* value)	*hif-1* (*ia04*) H/N ratio (*p* value)	*vhl-1* (*ok161*) H/N ratio (*p* value)	HRE[b]
fmo-12	Flavin-containing monooxygenase family member	6.31 (0.035)	0.78 (0.381)	1.50 (0.53)	
W07A12.6	Predicted acyltransferase containing T on B-box	6.14 (0.005)	0.80 (0.537)	0.93 (0.74)	2
F22B5.4	Unknown function	6.02 (0.003)	1.21 (0.646)	1.24 (0.58)	4
W07A12.7	Predicted acyltransferase	5.58 (0.005)	0.81 (0.531)	1.21 (0.83)	3
phy-2	Prolyl 4-hydroxylase αsubunit/collagen synthesis	4.85 (0.005)	1.18 (0.641)	1.42 (0.32)	3
Y44A6C.1	Unknown function	4.59 (0.031)	1.83 (0.300)	1.97 (0.20)	1
efk-1	Homologous to eEF-2 kinase	4.21 (0.004)	1.74 (0.122)	1.98 (0.69)	1
nhr-57	Nuclear hormone receptor family member	4.09 (0.004)	1.80 (0.093)	1.72 (0.10)	4
F59B10.4	Unknown function	3.89 (0.007)	1.81 (0.100)	1.47 (0.24)	1
egl-9	Dioxygenase that negatively regulates *hif-1*	3.70 (0.003)	1.11 (0.796)	1.74 (0.088)	1
F45D11.16	Unknown function	3.42 (0.030)	0.98 (0.818)	1.62 (0.43)	
C24B9.9	Unknown function	3.24 (0.016)	1.24 (0.658)	1.56 (0.27)	1
dpf-6	Dipeptidyl peptidase IV family member	3.18 (0.009)	0.47 (0.029)	0.91 (0.52)	1
W04E12.4	Unknown function	3.17 (0.008)	1.72 (0.114)	2.50 (0.025)	
C31G12.2	Contains domain found in C-type lectins	3.16 (0.015)	1.54 (0.228)	2.46 (0.031)	
Y38E10A.23	Unknown function	3.12 (0.038)	1.78 (0.159)	2.74 (0.03)	1
R10D12.1	Sodium/phosphate transporter	3.11 (0.038)	1.40 (0.446)	0.79 (0.46)	
K10H10.2	Cysteine synthase	3.07 (0.026)	0.92 (0.677)	1.18 (0.61)	3
npp-6	Predicted nuclear pore complex protein	3.05 (0.030)	1.62 (0.238)	1.96 (0.17)	
C34D4.14	Predicted ubiquitin-protein ligase	3.04 (0.016)	1.01 (0.969)	1.47 (0.24)	
Y15E3A.5	Unknown function	3.00 (0.016)	1.67 (0.181)	2.59 (0.022)	
inx-2	Innexin/putative gap junction protein	2.98 (0.019)	0.73 (0.244)	1.10 (0.83)	
K04H4.2	Predicted extracellular chitin-binding protein	2.98 (0.018)	0.63 (0.123)	0.58 (0.091)	
C04A2.1	Protein containing RabGAP/TBC domain	2.97 (0.006)	1.10 (0.755)	1.06 (0.85)	1
npc-1/*ncr-1*	Transmembrane protein orthologous to human NPC1	2.96 (0.002)	1.56 (0.084)	1.67 (0.050)	
T25E12.4	Serine/threonine protein kinase	2.92 (0.000)	1.23 (0.220)	2.00 (0.004)	1
M01H9.3	Unknown function	2.89 (0.016)	1.53 (0.300)	1.94 (0.095)	2
F45D11.1	Protein containing DUF684 domain	2.87 (0.039)	0.90 (0.750)	2.07 (0.10)	1
CC8.2	Putative phosphatase regulatory subunit	2.85 (0.004)	1.37 (0.206)	1.93 (0.023)	
K02E7.6	Protein containing DUF684 domain	2.71 (0.025)	1.02 (0.944)	1.31 (0.48)	
fmo-14	Homologous to hepatic flavin-containing monooxygenase 5	2.66 (0.010)	1.18 (0.679)	1.12 (0.69)	1
K08E3.1	Tyrosinase/ predicted oxidoreductase activity	2.64 (0.002)	1.26 (0.326)	1.96 (0.010)	4
F45D11.2	Protein containing DUF684 domain	2.56 (0.023)	0.94 (0.691)	1.73 (0.11)	
F16G10.10	Protein containing DUF130 domain	2.46 (0.006)	1.28 (0.332)	2.22 (0.010)	
C56E6.2	Ras GTPase superfamily member	2.43 (0.021)	1.49 (0.265)	1.76 (0.11)	1
smg-2	Regulator of nonsense-mediated mRNA decay	2.38 (0.029)	1.11 (0.780)	1.15 (0.77)	
cam-1	Receptor tyrosine kinase orthologous to human ROR1, ROR2	2.37 (0.004)	0.82 (0.290)	0.86 (0.32)	
F57B9.1	Putative pyridoxamine-phosphate oxidase	2.36 (0.002)	1.10 (0.708)	0.97 (0.70)	
F21D12.3	Putative transmembrane amino acid transporter protein	2.35 (0.003)	0.92 (0.474)	1.20 (0.40)	2
T08G3.6	Unknown function	2.34 (0.007)	1.51 (0.134)	1.85 (0.034)	
pqn-43	Large protein containing prion-like (Q/N-rich) domains	2.32 (0.044)	1.14 (0.778)	1.50 (0.27)	3
M02F4.1	Unknown function	2.32 (0.019)	0.78 (0.291)	1.29 (0.54)	2
Y46G5A.6	Protein containing F-box and helicase domains	2.28 (0.015)	1.50 (0.125)	1.37 (0.21)	
zyg-9	Spindle pole component/regulates microtubule assembly	2.28 (0.016)	1.12 (0.643)	1.61 (0.11)	1
M05D6.5	Protein containing hypoxia-induced protein conserved region	2.28 (0.009)	1.06 (0.897)	1.46 (0.16)	
C34B7.3	Cytochrome P450	2.28 (0.001)	0.79 (0.134)	1.09 (0.75)	1
str-257	Member of the seven-transmembrane receptor gene class	2.25 (0.017)	1.31 (0.299)	1.88 (0.043)	
T28F3.1	Protein containing copine and C2 domains	2.23 (0.038)	1.57 (0.250)	2.56 (0.053)	1
vab-1	Eph receptor tyrosine protein kinase	2.18 (0.004)	1.50 (0.052)	2.26 (0.003)	
Y55F3BR.4	Predicted extracellular ligand-gated ion channel	2.17 (0.016)	1.26 (0.439)	1.39 (0.21)	1
let-75	Pharynx-specific type II myosin heavy chain (MHC D)	2.17 (0.039)	0.65 (0.087)	0.76 (0.32)	
C40D2.4	Homeobox-containing protein	2.16 (0.022)	1.08 (0.939)	1.55 (0.14)	
C29F9.5	Similarity to p300 transcriptional coactivator	2.15 (0.024)	1.42 (0.227)	2.25 (0.022)	1
C36A4.9	Putative acetyl-coenzyme A synthetase	2.15 (0.021)	0.96 (0.790)	1.87 (0.063)	
K10H10.4	Unknown function	2.13 (0.003)	1.25 (0.221)	1.37 (0.14)	
C32D5.12	Similarity to sterol dehydrogenases	2.11 (0.017)	1.36 (0.332)	1.04 (0.85)	
Y19D10A.5	Transporter belonging to major facilitator superfamily	2.10 (0.004)	0.84 (0.244)	0.91 (0.48)	
C01G6.9	Limited homology to human cetriolin	0.36 (0.008)	1.03 (0.748)	1.20 (0.37)	
C41C4.1	Protein containing DUF595 domain	0.41 (0.043)	0.68 (0.622)	1.06 (0.80)	1
F35D11.10	Contains domain found in C-type lectins	0.43 (0.016)	0.83 (0.405)	0.93 (0.75)	
T07D1.3	Aldehyde dehydrogenase domain	0.47 (0.044)	1.23 (0.374)	2.56 (0.010)	
Y9C9A.12	Protein containing F-box domain	0.46 (0.007)	0.78 (0.222)	0.91 (0.92)	
F13H8.4	Unknown	0.48 (0.022)	0.71 (0.284)	0.84 (0.60)	

[a] H/N = mRNA expression in a hypoxic condition relative to mRNA levels in a normoxic condition.
[b] HRE = 5′-TACGTG, a motif similar to mammalian HRE, in the predicted 5′-regulatory region of the gene.

Although the hypoxia-responsive genes are located on all six chromosomes, 40% of the *hif-1*-dependent genes are on chromosome II, and many of these are found in clusters (Fig. 2). Three genes (F45D11.1, F45D11.2, and F45D11.16) are within 50 kb of each other at the left arm of II. Multiple small clusters are located at the center of the chromosome, and a 10-kb domain on the right arm of II contains two *hif-1*-dependent genes (K10H10.2 and K10H10.4). With one exception (two *hif-1*-independent genes on chromosome III), hypoxia-responsive genes on the other four autosomes or on the X are not clustered.

We anticipated that some of the *hif-1*-dependent genes would be direct targets of the HIF-1 transcriptional complex. The mammalian HIF-1α and β subunits form a heterodimer and bind to specific DNA sequences (HREs) that contain the sequence 5′-RCGTG (27). A survey of the sequences 200–2,000 bp 5′ to the predicted translational start sites shows that the motif

TABLE II
47 genes regulated by hypoxia independently of hif-1 function

Gene	Description	Wild type H/N ratio[a] (*p* value)	*hif-1* (*ia04*) H/N ratio (*p* value)	*vhl-1* (*ok161*) H/N ratio (*p* value)
F26A3.4	Protein containing dual specificity phosphatase domain	10.04 (0.004)	4.71 (0.029)	5.13 (0.015)
F26H11.3	Predicted transcription factor containing a bromodomain	8.76 (0.019)	4.69 (0.048)	6.41 (0.035)
C12C8.1	Member of the Hsp70 family of heat shock proteins	5.60 (0.025)	11.73 (0.009)	11.34 (0.005)
F08H9.4	Member of the Hsp16 family of heat shock proteins	5.28 (0.016)	6.79 (0.004)	2.1 (0.14)
F44E5.5	Member of the Hsp70 family of heat shock proteins	4.87 (0.027)	7.66 (0.012)	17.3 (0.001)
R166.5	Protein containing serine/threonine kinase domain	4.52(0.023)	3.54 (0.042)	3.16 (0.050)
F44E5.4	Member of the Hsp70 family of heat shock proteins	4.49 (0.038)	6.45 (0.021)	15.06 (0.001)
F45D3.4	Unknown function	3.06 (0.040)	4.18 (0.030)	1.73 (0.021)
W02D9.10	Unknown function	3.02 (0.007)	3.80 (0.004)	2.71 (0.014)
C07D10.2	Protein containing BTB/ POZ protein binding domain	2.99 (0.001)	1.74 (0.022)	1.65 (0.036)
bli-3	NADPH oxidase required for dityrosine cross-linking of collagen	2.92 (0.0096)	1.77 (0.048)	1.54 (0.15)
M04G7.3	Unknown function	2.91 (0.008)	1.97 (0.036)	1.89 (0.037)
gei-7	Predicted isocitrate lyase/malate synthase	2.82 (0.015)	2.87 (0.027)	2.54 (0.026)
tbx-33	T-box family transcription factor	2.77 (0.002)	1.94 (0.016)	2.39 (0.007)
Y41D4B.22	Unknown function	2.72 (0.006)	2.06 (0.025)	2.98 (0.005)
Y22D7AR.1	Predicted protein-tyrosine phosphatase	2.60 (0.018)	2.00 (0.042)	3.29 (0.009)
F58D5.8	Unknown function	2.55 (0.004)	2.14 (0.009)	2.40 (0.006)
ZK470.2	Unknown function	2.54 (0.008)	1.93 (0.027)	2.12 (0.019)
ptr-8	Member of the patched related family of transmembrane glycoproteins	2.51 (0.016)	2.76 (0.012)	1.78 (0.067)
Y39G8B.2	Member of aldo/ketoreductase family	2.49 (0.006)	2.10 (0.013)	2.55 (0.007)
Y75B8A.3	Predicted carboxylesterase	2.47 (0.024)	1.75 (0.013)	3.70 (0.005)
H23N18.3	Similar to UDP glucuronosyltransferases	2.40 (0.024)	2.04 (0.036)	1.48 (0.22)
Y75B8A.6	Predicted DNA-binding protein with two zinc finger domains	2.39 (0.006)	2.24 (0.010)	2.52 (0.007)
sca-1	Sarco/endoplasmic reticulum Ca^{2+} ATPase homolog; ortholog of human ATP2A1	2.32 (0.031)	2.73 (0.044)	1.60 (0.17)
Y39G10AR.3	Protein containing RCC1 (regulator of chromosome condensation) domain	2.31 (0.017)	2.23 (0.024)	2.87 (0.007)
lin-42	Homolog of period PAS domain protein	2.29 (0.024)	2.34 (0.033)	2.60 (0.017)
F38B7.2	Unknown function	2.26 (0.021)	2.53 (0.010)	1.51 (0.17)
Y75B8A.19	Unknown function	2.26 (0.023)	2.50 (0.021)	2.24 (0.031)
F47C12.2	Predicted extracellular protein containing lectin and CUB domains	2.25 (0.024)	1.88 (0.045)	2.86 (0.012)
pyc-1	Orthologous to the human gene pyruvate carboxylase	2.24 (0.038)	2.66 (0.018)	2.40 (0.018)
K03B4.4	Unknown function	2.21 (0.011)	1.79 (0.049)	1.86 (0.044)
nhr-88	Member of the nuclear hormone receptor family	2.21 (0.019)	1.96 (0.048)	2.76 (0.008)
unc-14	Protein that binds the UNC-51 protein kinase	2.21 (0.002)	1.78 (0.009)	1.87 (0.006)
F30B5.4	Unknown function	2.20 (0.004)	1.93 (0.11)	2.30 (0.004)
K02F6.7	Unknown function	2.20 (0.012)	1.83 (0.045)	2.90 (0.004)
mxl-3	Basic helix-loop-helix family of DNA-binding proteins; orthologous to human MAX	2.18 (0.018)	2.39 (0.011)	2.74 (0.009)
E03H4.12	Predicted glucuronyltransferase	2.18 (0.004)	1.94 (0.011)	2.16 (0.005)
tax-6	Ortholog of calcineurin A	2.16 (0.015)	1.92 (0.033)	1.7 (0.047)
T16H12.5	Protein containing MATH and BTB/POZ protein binding domains	2.14 (0.017)	1.86 (0.047)	1.27 (0.37)
ZK849.1	Protein containing PDZ domain	2.12 (0.006)	1.61 (0.036)	1.71 (0.019)
F40E12.2	Large intracellular protein with ankyrin and BRCT domains	2.11 (0.022)	1.76 (0.041)	2.58 (0.009)
F18A12.8	Encodes neprilsin; cleaves small signaling peptides	2.11 (0.000)	2.12 (0.000)	2.40 (0.0002)
tbx-38	T-box family transcription factor	2.07 (0.027)	2.41 (0.018)	2.22 (0.023)
mod-5	Sodium chloride-dependent serotonin transporter	2.05 (0.008)	1.61 (0.043)	2.03 (0.010)
ins-22	Predicted type-α insulin-like molecule	0.28 (0.024)	0.42 (0.132)	0.35 (0.045)
T12B5.13	Protein containing FTH protein interaction domains	0.41 (0.023)	0.50 (0.049)	0.63 (0.21)
srv-22	Member of the class V serpentine receptor family	0.42 (0.002)	0.64 (0.030)	0.57 (0.016)

[a] H/N = mRNA expression in a hypoxic condition relative to mRNA levels in a normoxic condition.

5′-TACGTG is present in 46% of the *hif-1*-dependent genes (Table I). In comparison, this putative HRE is present in only 23% of *hif-1*-independent genes (Table II).

Functions of Hypoxia-responsive Genes—Metabolic enzymes are prevalent in both Table I and Table II. For example, *pyc-1* encodes pyruvate carboxylase, a key enzyme in gluconeogenesis, and it was induced by hypoxia in an *hif-1*-independent manner (2.2-fold; *p* value 0.038). Some glycolytic enzymes were induced at lower levels. F14B4.2 hexokinase, R05F9.6 phosphoglucomutase, T21B10.2 enolase, and R11F4.1 glycerol kinase are expressed 1.4–1.9-fold higher levels in hypoxia relative to normoxia ($p < 0.05$). Two enzymes predicted to facilitate the conversion of fatty acids to sugars were also induced by hypoxia. C05E4.9/*gei-7* (isocitrate lyase) and F54H12.1/*aco-2* (aconitate hydratase) were induced 2.8-fold and 1.8-fold, respectively (Table II and supplemental data).

Predicted signaling molecules are another major functional class in both Tables I and II. For example, F26A3.4, an *hif-1*-independent gene, encodes a protein-tyrosine phosphatase similar to the mammalian mitogen-activated protein kinase phosphatase 6. Additionally, *cam-1*, a receptor tyrosine kinase orthologous to human ROR1 and ROR2, and the *dpf-6* dipeptidyl peptidase, which is predicted to hydrolyze peptide ligands, are both *hif-1*-dependent genes.

Hypoxia appeared to induce changes in extracellular matrices. The *phy-2* prolyl 4-hydroxylase α subunit gene and the *bli-3* NADPH oxidase were induced by hypoxia, and both have central roles in collagen synthesis (28, 29) (Tables I and II, respectively). Genes encoding three predicted extracellular molecules with lectin domains (C31G12.2, F35D11.10, and F47C12.2) and one predicted chitin-binding protein (K04H4.2) also exhibited hypoxia-responsive mRNA expression (Tables I and II).

Interestingly, we found that *egl-9* mRNA levels were induced by hypoxia in an *hif-1*-dependent manner (Fig. 1*A*). The *egl-9* gene encodes a 2-oxoglutarate-dependent oxygenase, and it is thought to be the sole HIF prolyl hydroxylase (PHD) in *C. elegans*. Using oxygen as a substrate, EGL-9

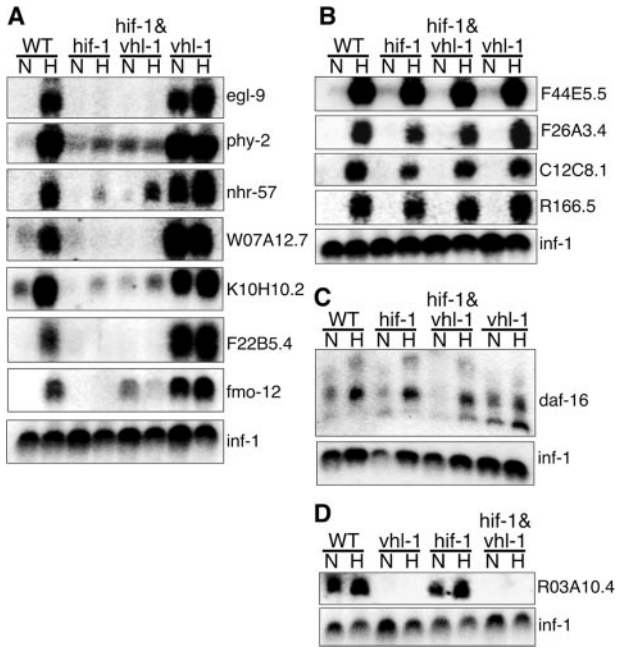

FIG. 1. **RNA blot analyses of genes regulated by *hif-1* or *vhl-1*.** mRNA was isolated from wild-type (*WT*), *hif-1* (*ia04*), *vhl-1* (*ok161*), or double mutant animals after incubation in 21% oxygen (*N*) or 0.5% oxygen (*H*) for 4 h at 21 °C. *inf-1* serves as a loading control.

hydroxylates HIF-1 in the conserved L*XX*LAP motif. This modification increases the affinity of HIF-1 for the VHL-1 E3 ligase, which targets HIF-1 for proteosomal degradation. In *egl-9*-deficient animals, HIF-1 protein is expressed at constitutively high levels (6).

hif-1 function was required for hypoxia-dependent regulation of *smg-2, efk-1*, and a predicted ubiquitin ligase (C34D4.14) (Table I). These gene products are predicted to act post-transcriptionally to change cellular proteosomes. *smg-2* is required for the degradation of mRNAs containing early nonsense codons. The gene *efk-1* is strongly induced by hypoxia, and it encodes a calcium/calmodulin-dependent kinase with homology to human elongation factor-2 kinase (eEF-2 kinase). eEF-2 kinase decreases protein synthesis by phosphorylating and inactivating elongation factor 2. Mammals activate eEF-2 kinase activity upon oxygen deprivation (30). Thus, modulation of protein synthesis via eEF-2 kinase may be an important aspect of adaptation to changing oxygen levels.

Interestingly, many of the *hif-1*-independent genes listed in Table II are transcription factors and heat shock proteins. 17% (8/47) of *hif-1*-independent genes are predicted transcriptional regulators. In comparison, only 5% (3/63) of the *hif-1*-dependent genes are likely transcription factors, and this is similar to the prevalence of predicted transcription factors in the complete genome (31). The *C. elegans* heat shock protein genes C12C8.1, F08H9.4, F44E5.5, and F44E5.4 were all induced 4.5–5.6-fold by hypoxia, and induction of these heat shock proteins does not require *hif-1* function (Table II).

Many other *C. elegans* researchers have performed microarray experiments to identify genes that were expressed differentially during specific developmental stages or in response to diverse environmental stresses. Computational analyses of these data have resulted in the identification of "mountains" of coexpressed genes (32). We note that 43% of the *hif-1*-independent (20/47) and 13% of the *hif-1*-dependent genes (8/63) are in "mountain 1," an 1,818-gene cluster enriched in genes with neuronal or muscle function.

Essential Functions for Individual hif-1-dependent Genes—Mutants lacking *hif-1* function exhibit a much higher level of embryonic and larval lethality in hypoxic conditions compared with wild-type *C. elegans*. Thus, we anticipated that certain individual HIF-1 target genes might also be critical for survival in hypoxia. To test this hypothesis, we used existing mutant strains or RNAi to examine the requirement for 16 individual *hif-1*-dependent genes in hypoxia. We tested 7 strains carrying loss-of-function mutations in *hif-1*-dependent genes, and 5 of the strains exhibited a statistically significant decrease in embryonic or larval viability in 0.5% oxygen relative to wild-type animals. As shown in Fig. 3, animals carrying loss-of-function mutations in *fmo-12*, *fmo-14*, *vab-1*, or *inx-2* were slightly more vulnerable to hypoxic stress than were wild-type worms.

The most dramatic hypoxia-dependent phenotypes were evident in animals carrying a strong loss-of-function mutation in the *phy-2* prolyl 4-hydroxylase α subunit. Prior studies have shown that the *phy-2* prolyl 4-hydroxylase α subunit has a central role in collagen synthesis, and genetic analyses have shown that in normal culture conditions the developmental functions of *phy-2* could also be provided by *dpy-18* (23, 33). As shown in Fig. 3, only 22.3% of *phy-2* (*ok177*) mutant animals successfully completed embryogenesis in 0.5% oxygen. 97% of wild-type animals and 58.2% of *hif-1* (*ia04*) embryos hatched in the same conditions. Animals lacking *dpy-18* function were relatively healthy in hypoxia (94% hatch; $n = 91$). These data show that *dpy-18* cannot completely compensate for loss of *phy-2* function when oxygen is limiting.

When *phy-2* (*ok177*) mutants were allowed to complete embryogenesis in normal culture conditions and then transferred to a hypoxic chamber (0.5% oxygen) to complete development, 79% were sterile as adults ($n = 38$). This was unexpected because expression of a *phy-2*:lacZ transgene has been reported to be predominantly hypodermal (23). To examine the *phy-2* expression pattern in live animals, we constructed a *phy-2:*GFP reporter. *phy-2:*GFP was strongly expressed in the hypodermis, excretory cell, and spermatheca (Fig. 4). These data are consistent with the hypothesis that collagen synthesis in the spermatheca is important to fertility and embryonic viability.

We used RNAi to examine the requirement for an additional 9 *hif-1*-dependent genes. These assays required culturing the animals on plates containing isopropyl 1-thio-β-D-galactopyranoside and tetracycline, and worms grown on control bacteria in these conditions showed increased sensitivity to hypoxia (Fig. 5). Worms treated with RNAi for the genes F22B5.4 (unknown function), *nhr-57* (nuclear hormone receptor), K10H10.2 (cysteine synthase), or M05D6.5 (protein containing a hypoxia-inducible domain) exhibited higher levels of embryonic lethality in 0.5% oxygen (Fig. 5).

Molecular Links between Hypoxia Response and Dauer Formation—Some of the genes induced by hypoxia also regulate entry into the dauer phase, an alternative larval stage characterized by low metabolic rate and resistance to environmental stress. Mutations that favor dauer formation generally also confer longevity and resistance to certain environmental stresses. At sufficiently high temperatures, oxygen deprivation is toxic to wild-type *C. elegans,* and this experimental regimen has been termed hypoxic death. Interestingly, specific mutations in the *daf-2* insulin-like growth factor receptor gene confer resistance to hypoxic death (34). Loss-of-function mutations in *daf-2* also cause constitutive dauer formation and increased longevity. These phenotypes are suppressed by mutations that decrease the function of DAF-16, a forkhead transcription factor (35, 36). In our microarray experiments, *daf-16* mRNA was induced 1.7–1.9-fold by hypoxia in wild-type, *hif-1* (*ia04*), or *vhl-1* (*ok161*) worms ($p < 0.02$ to $p < 0.05$) (supplemental data). We performed RNA blot analyses to validate these findings, and these experiments showed that expression of *daf-16* mRNA

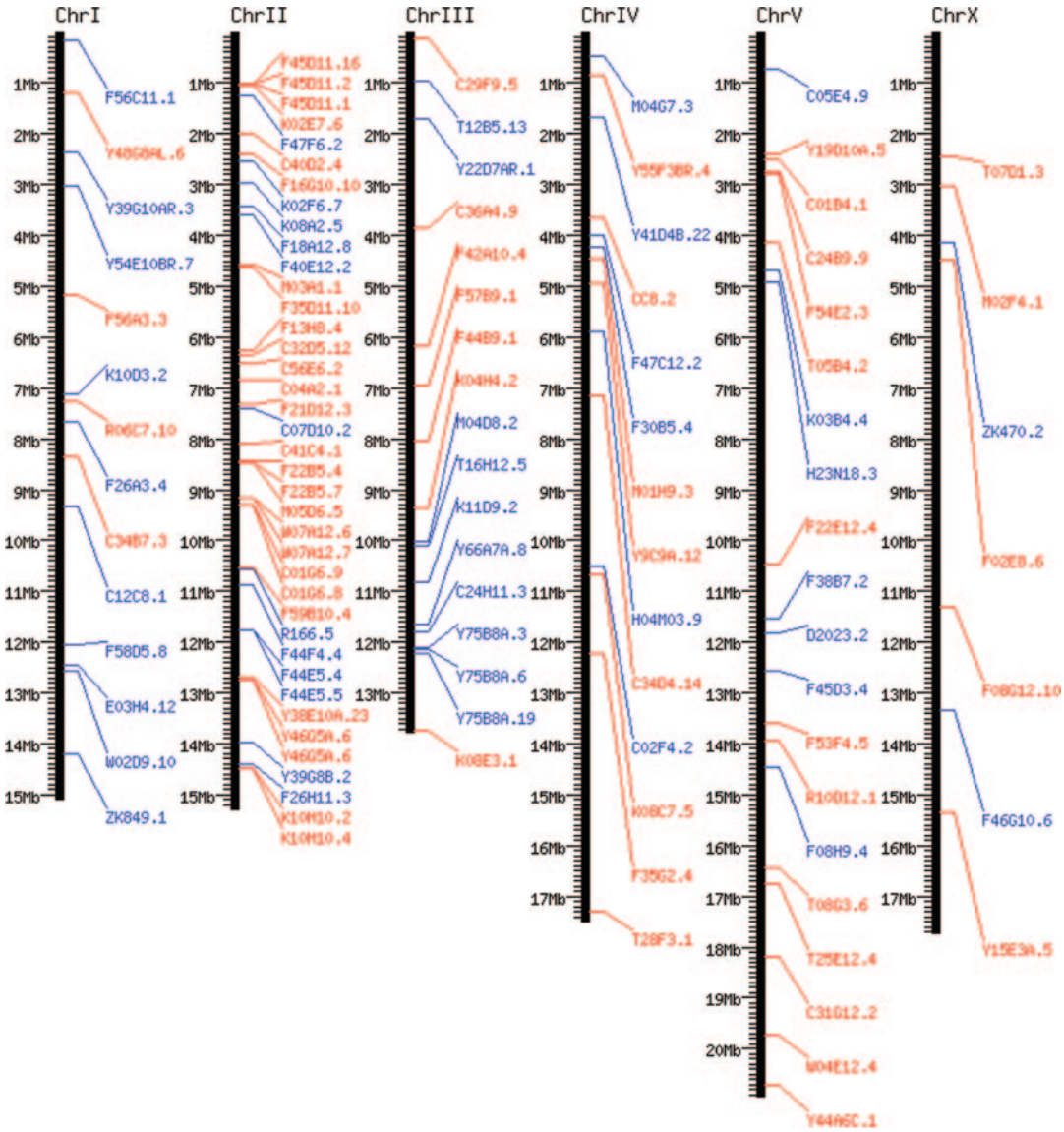

FIG. 2. **Genomic distribution of hypoxia-responsive genes.** Genes that are induced by hypoxia in wild-type, but not *hif-1* (*ia04*) animals, are listed in *red*. Genes induced by hypoxia independently of *hif-1* function are listed in *blue*.

was ~2-fold higher in worms treated with 0.5% oxygen for 4 h compared with normoxia-treated worms (Fig. 1*C*). Thus, increased *daf-16* expression is a common feature of both hypoxia response and of perturbations in the DAF-2 signaling pathway which lead to constitutive dauer formation, increased longevity, and enhanced stress resistance (Fig. 6).

The *hif-1*-dependent genes in Table I include the *npc-1* NPC1 homolog and the *cam-1* receptor tyrosine kinase. Both of these genes have been shown to regulate dauer formation negatively. The human NPC1 gene is structurally related to the patched family of receptors, and it regulates intracellular cholesterol transport. Loss of human NPC1 function is associated with Niemann-Pick type C disease. The *C. elegans* genome encodes two NPC1 homologs, and deletion of either gene results in slow growth and hypersensitivity to cholesterol deprivation. Inactivation of both *npc-1* and *npc-2* causes a constitutive dauer phenotype (37, 38). Other studies have shown that mutants lacking the *cam-1* kinase domain form dauers constitutively when they are cultured at 27 °C, a temperature slightly higher than the normal physiological range for *C. elegans* (39). This temperature-dependent phenotype was especially intriguing because *hif-1* (*ia04*) mutants are unable to acclimate to high temperatures (40). Collectively, these data suggested that *hif-1*-defective worms might exhibit a dauer constitutive phenotype at high temperatures. We tested this hypothesis and determined that *hif-1* (*ia04*) mutants grew slower than wild-type worms at 27 °C, and 98% of the *hif-1* mutants arrested as partial dauers (n = 236). Upon arrest, the *hif-1* (*ia04*) partial dauers were thin; they pumped their pharynxes slowly and sporadically; and they had thick cuticles.

VHL-1 Regulates HIF-1 Function and Other Unknown Pathway(s)—Prior studies had demonstrated that *C. elegans* lacking *vhl-1* function express HIF-1 protein at constitutively high levels (6). Thus, we predicted that hypoxia-dependent changes in gene expression induced by HIF-1 would be abrogated in *vhl-1*-defective mutants but that inactivation of *vhl-1* would have little effect on *hif-1*-independent genes. Our data generally validate these models. For 60 of the 63 *hif-1*-dependent genes, regulation by hypoxia was diminished in *vhl-1* (*ok161*) mutants, and 50 of the genes did not show a significant difference ($p > 0.05$) between mRNA levels in hypoxia and normoxia. In contrast, 39 of the 47 genes in Table II exhibited significant regulation by hypoxia in *vhl-1* mutants.

Close examination of the raw data also provided evidence that VHL has *hif-1*-independent functions. The gene R03A10.4 (predicted glutamine-phenylpyruvate transaminase) was not

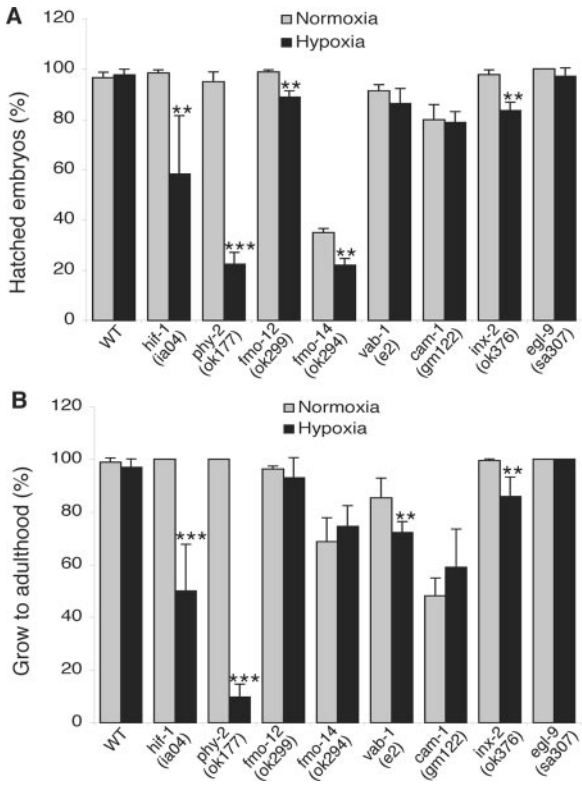

FIG. 3. **Viability assays in normoxia (21% oxygen) and hypoxia (0.5% oxygen).** Animals carrying strong loss-of-function mutations in downstream targets of HIF-1 were incubated in 0.5% oxygen (hypoxia) or room air (normoxia) for 24 h and then assayed for successful completion of embryogenesis (*A*) and survival to adulthood as described previously (16) (*B*). Numerical values are listed in Table S2 (supplemental data). *Asterisks* indicate that the viability of hypoxia-treated mutants is significantly lower than that of wild-type (*WT*).

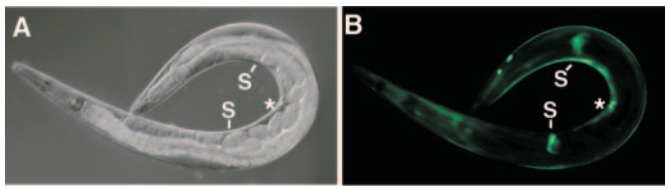

FIG. 4. ***phy-2*:GFP expression in an adult hermaphrodite.** The positions of the vulva and the spermathecas are indicated with a *star* and an *s*, respectively.

regulated by hypoxia, and mutation of *hif-1* had little effect on its expression. However, animals lacking *vhl-1* function expressed R03A10.4 at nearly undetectable levels (Fig. 1*D*). Thus, VHL-1 appears to act through an HIF-1-independent mechanism to positively regulate the expression of R03A10.4.

DISCUSSION

Cellular response to changing environmental levels of oxygen is critical for the survival of all metazoans. This study represents the first genomewide analysis of hypoxia response in *C. elegans* and demonstrates that *hif-1* function is required for the majority of mRNA expression changes in *C. elegans* hypoxia response. We have also presented compelling evidence for one or more HIF-1-independent pathways for implementing transcriptional responses to low oxygen.

In humans response to hypoxia plays a central role in angiogenesis, tumor development, and cardiovascular disease. The hypoxia-inducible factor is thought to be the most important transcriptional regulator of mammalian hypoxic response during normal development and disease states (1, 14). Studies from other research groups have identified many genes regu-

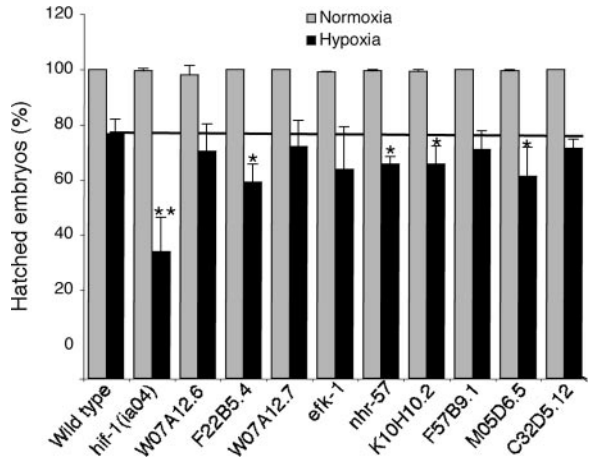

FIG. 5. **Bacteria-mediated RNAi of nine downstream targets of HIF-1.** The percentage of *C. elegans* that completed embryogenesis and hatched is shown. Numerical values are listed in Table S3 (supplemental data).

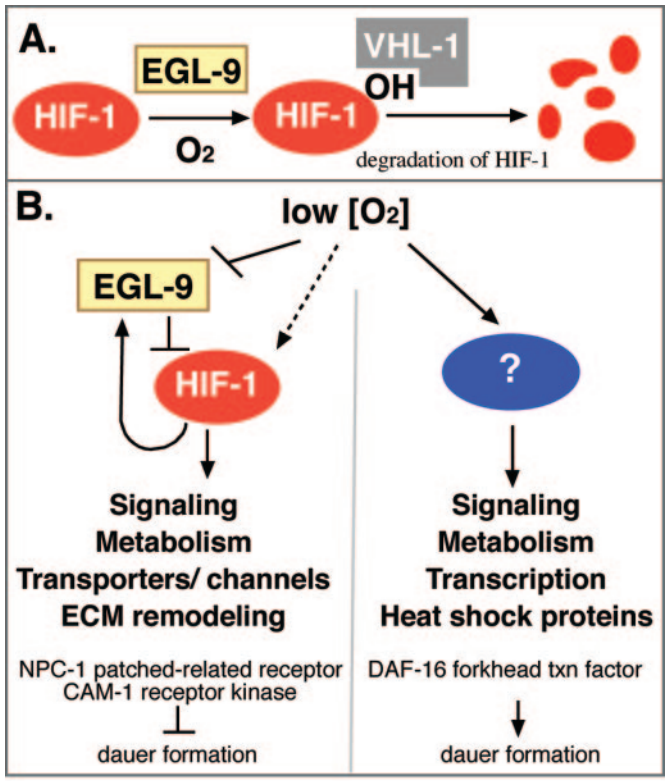

FIG. 6. **Models for hypoxia signaling and response.** *A*, proteasomal degradation of HIF-1 is mediated by EGL-9 and VHL-1 (6). *B*, response to hypoxia is mediated by HIF-1 and by HIF-1-independent pathway(s). The four largest functional categories of genes induced by each pathway are shown. HIF-1 positively regulates the expression of *npc-1* and *cam-1*, which are negative regulators of dauer formation. This is consistent with constitutive dauer formation of *hif-1* (*ia04*) mutants at 27 °C. *daf-16* mRNA expression is induced by hypoxia independently of *hif-1* function.

lated by HIF-1. Some of the genes that are transcriptionally activated by mammalian HIF-1 induce systemic responses to oxygen deprivation, such as increased red blood cell production and angiogenesis. Other HIF-1 targets modify the extracellular matrix, regulate glucose metabolism, or contribute to cellular survival in stressful conditions (41).

Consistent with studies of mammalian HIF-1 function and with physiological studies of *C. elegans* oxygen utilization, we found that hypoxia induced the expression of many genes that

regulate cellular metabolism. Adult *C. elegans* do not have a complex circulatory system and are ~1 mm in length. Any cell in the worm is only a few cell widths from the outer cuticle or the intestinal lumen (42), and oxygen delivery is likely accomplished by diffusion. Thus, we anticipated that the transcriptional targets of the *C. elegans* HIF-1 complex would regulate cell-autonomous responses to oxygen deprivation. *C. elegans* can maintain near normal metabolic rates at environmental oxygen concentrations as low as 2% (25), and wild-type animals survive oxygen concentrations as low as 0.1% (26). In hypoxia (0.1–1% oxygen), *C. elegans* have been shown to decrease feeding, movement, and oxygen consumption (25).

The Oxygen-requiring PHDs phy-2 and egl-9 are hif-1-dependent Genes—The prolyl 4-hydroxylase enzymes that modify collagen in the endoplasmic reticulum require oxygen as a substrate. *C. elegans* has been shown to encode three prolyl 4-hydroxylase α subunits. The expression pattern of *phy-2* overlaps with that of two other α subunits, *phy-1* and *phy-3*, in the hypodermis and in the spermatheca, respectively. The majority of the proteins in the worm cuticle are collagens, and double mutants lacking *phy-1* and *phy-2* function are inviable (23, 33, 43). As shown in Fig. 1, *C. elegans phy-2* mRNA was induced by hypoxia in an *hif-1*-dependent manner. Transcriptional regulation of the prolyl 4-hydroxylase α subunits by HIF appears to be evolutionarily conserved (44). We have shown that *phy-2*-defective animals exhibit lethality and sterility in hypoxic conditions. These data fit a model in which *phy-2* function, and perhaps increased transcription of *phy-2*, is required to offset decreased availability of oxygen substrate in hypoxic conditions.

The EGL-9 PHD modifies HIF-1, thereby increasing its affinity for the VHL-1 E3 ligase that targets HIF-1 for proteosomal degradation (6). Data in Table I and Fig. 1 show that *egl-9* mRNA levels are regulated by *C. elegans hif-1*. This is in agreement with a recently published independent study (42). Positive regulation of *egl-9* transcription by HIF-1 should help to maintain EGL-9 activity when oxygen substrate is limiting, and it may create a negative feedback loop to attenuate HIF-1 activation (Fig. 6). In humans, three PHD genes (EGL-9 homologs) have been identified, and PHD2 and PHD3 are induced by hypoxia (6, 45, 46). Thus, this regulatory mechanism is evolutionarily conserved.

In mammals, HIF-1 also induces the expression of CITED2, which competes with CBP/p300 for binding to HIF-1, thereby inhibiting transcriptional activation by HIF-1. HIF-1 transcriptional repressors have not yet been identified in *C. elegans*. The *C. elegans* homologs of HIF-1 and CBP have been shown to bind each other in yeast two-hybrid assays (47), but it is not yet known whether interaction of the native proteins is regulated by environmental oxygen concentrations.

hif-1-independent Pathway(s) for Hypoxia Response—Relatively little is known about the oxygen sensors and regulatory pathways that mediate transcriptional responses independent of HIF. The data presented in Table II and Fig. 1*B* clearly demonstrate that there are *hif-1*-independent mechanisms for hypoxia adaptation in *C. elegans*. Consistent with these findings, others have shown recently that the transcription of certain *hsp16* small heat shock proteins is induced by hypoxia, and this induction does not require *hif-1* function (48).

For all 47 of the *hif-1*-independent genes, hypoxia causes significant differences in expression in the absence of *hif-1* function, but in some cases, such as the F26A3.4 phosphatase, the gene expression change is ~2-fold less than that seen in wild-type animals (Fig. 1*B*). This supports a model in which some genes are regulated by hypoxia via both *hif-1*-dependent and *hif-1*-independent pathways.

To compare hypoxia-induced changes in mRNA levels in different genetic backgrounds, we assayed animals from a defined developmental stage (L3) and at a single early time point. We expect that assays performed at other oxygen concentrations or other developmental stages will reveal additional hypoxia-responsive genes. Early HIF-1 targets likely control later responses to oxygen deprivation. 67 of the hypoxia-responsive genes in Tables I and II have known or predicted functions, and 26 of these are expected to mediate transcription or intracellular protein-protein interactions. For example, F26A3.4 (listed at the top of Table II) encodes a protein-tyrosine phosphatase similar to the mammalian mitogen-activated protein kinase phosphatase 6. Predicted transcription factors induced by hypoxia include the nuclear hormone receptors *nhr-57* and *nhr-88* and the T-box family members *tbx-33* and *tbx-38*.

Oxygen Levels and the Dauer Decision—Dauers are resistant to several types of stress, including oxygen deprivation (34). Recent analyses have also shown that the putative HIF-1 binding site (HRE) is overrepresented in the putative regulatory sequences of genes up-regulated in dauers (49). This had led to the hypothesis that HIF-1 may act in concert with DAF-16 (the forkhead transcription factor that promotes longevity and dauer formation) to regulate some of the genes that confer resistance to hypoxic stress (49). As illustrated in Figs. 1 and 6, hypoxia increases both *daf-16* expression and HIF-1 activity. Interestingly, some of the genes induced by HIF-1 inhibit dauer formation, and *hif-1*-defective animals form dauer-like larvae at high temperatures, even in the presence of abundant food. Because *hif-1* mutants are unable to acclimate to higher temperatures (40), dauer formation may allow survival of stressful conditions that might otherwise be lethal to animals that are not expressing HIF-1 target genes at sufficient levels.

Some responses to oxygen stress are post-translational. A recent study demonstrated that *C. elegans* will aerotax from 21% oxygen to 7% oxygen, and this response is mediated by *gcy-35*, which encodes an oxygen-sensing guanylate cyclase (50). *C. elegans* are standardly cultured in the laboratory in room air (21% oxygen). In the wild, *C. elegans* inhabits the soil, and the oxygen levels in soil containing ample supplies of its bacterial food source could be substantially below 21%. In future studies, it will be interesting to investigate whether the expression of hypoxia-responsive genes varies significantly between 21% oxygen and the lower oxygen levels preferred by *C. elegans*.

The microarray experiments reported here were not designed to compare gene expression patterns in *vhl-1* mutant and wild-type animals directly, but we did find one gene that was regulated by VHL-1 but not HIF-1. An independent study has recently been published which identified additional *C. elegans* genes regulated by *vhl-1* independently of *hif-1* (42). This is especially interesting because mutations have been identified in the mammalian VHL tumor suppressor protein which predispose individuals to carcinomas, but do not impair HIF-1α ubiquitination (51, 52). Further studies of HIF-1α and VHL homologs in *C. elegans* may shed light on the shared and distinct cellular pathways that regulate tumorigenesis.

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