THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 50, pp. 38675–38681, December 15, 2006

Induction of the Heat Shock Pathway during Hypoxia Requires Regulation of Heat Shock Factor by Hypoxia-inducible Factor-1*

Received for publication, August 21, 2006, and in revised form, October 11, 2006 Published, JBC Papers in Press, October 13, 2006, DOI 10.1074/jbc.M608013200

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Activation of heat shock proteins (Hsps) is critical to adaptation to low oxygen levels (hypoxia) and for enduring the oxidative stress of reoxygenation. Hsps are known to be regulated by heat shock factor (Hsf), but our results demonstrate an unexpected regulatory link between the oxygen-sensing and heat shock pathways. *Hsf* transcription is up-regulated during hypoxia due to direct binding by hypoxia-inducible factor-1 (HIF-1) to HIF-1 response elements in an *Hsf* intron. This increase in Hsf transcripts is necessary for full *Hsp* induction during hypoxia and reoxygenation. The HIF-1-dependent increase in Hsps has a functional impact, as reduced production of Hsps decreases viability of adult flies exposed to hypoxia and reoxygenation. Thus, HIF-1 control of *Hsf* transcriptional levels is a regulatory mechanism for sensitizing heat shock pathway activity in order to maximize production of protective Hsps. This cross-regulation represents a mechanism by which the low oxygen response pathway has assimilated complex new functions by regulating the key transcriptional activator of the heat shock pathway.

In order to endure oxygen deprivation, most eukaryotes utilize a conserved set of cellular adaptations (1). Many of these changes are brought about by the activation of the transcription factor hypoxia-inducible factor-1 (HIF-1),[2] a heterodimeric complex composed of HIF-1α and HIF-1β subunits. When this complex is formed it binds to specific DNA enhancer sequences and regulates the activity of target genes. Both HIF-1α and HIF-1β are constitutively expressed in normal oxygen conditions (normoxia), but HIF-1α protein is quickly degraded before dimerization can occur with HIF-1β (2). Normoxic HIF-1α degradation is mediated by a series of hydroxylations and ubiquitinations that tag HIF-1α for disposal through the proteasome (3–6).

The HIF-1 complex transcriptionally regulates a wide array of genes involved in anaerobic metabolism, growth, proliferation, angiogenesis, and cell death (7, 8). This multifaceted control of cellular and organismal physiological pathways is exploited by solid tumors through the natural hypoxic environment caused by rapid growth or genetic alterations that stabilize HIF-1α (9). Overexpression or activation of HIF-1α is often seen in a wide array of cancers and is correlated with patient survival (10), and studies have shown that targeting the HIF-1 pathway is a promising means of cancer therapy (11, 12). Thus, HIF-1 is a central regulator of normal and pathological changes in response to low oxygen.

Although many genes that are up-regulated during hypoxia are known to be regulated by HIF-1, there are also diverse sets of genes up-regulated that have not been linked to the actions of HIF-1. Among these are the highly conserved heat shock proteins (Hsps) that are highly up-regulated during hypoxia but have not been linked to HIF-1 regulation (13). Hsps are known to act as cellular chaperones for proteins that are misfolded by cellular stresses (14). Heat shock factor (Hsf) was one of the first studied transcription factors, and its activation by stresses that promote the unfolding of proteins has been well characterized. When cells are unstressed Hsf is in a monomeric state, but cellular stress induces trimerization of the protein (15, 16). The trimeric form of Hsf activates transcription of downstream genes such as *Hsps* (17, 18). However, this study identifies a novel mode of regulation of heat shock pathway activity during hypoxia through a HIF-1-dependent increase in Hsf transcript levels. This up-regulation of *Hsf* is necessary for the full increase of Hsp transcripts normally observed during hypoxia and also during reoxygenation. These findings establish a novel regulatory link between two stress pathways previously thought to be independent in responding to hypoxia.

EXPERIMENTAL PROCEDURES

Cell Culture and Hypoxia Treatments—Drosophila melanogaster Kc_{167} tissue culture cells were obtained from the Drosophila Genomics Resource Center. Cells were maintained in Schneider's *Drosophila* medium (Invitrogen) supplemented with 5% heat-inactivated fetal bovine serum (Invitrogen). For hypoxia experiments, cells were incubated for 6 h in chambers flushed with 0.5% O_2 gas. The reoxygenation step consisted of a 15-min return to normal oxygen levels.

RNA Interference (RNAi)—RNAi was performed as previously reported (19). The following primer pairs were used to generate template DNA: control green fluorescent protein (GFP) (5′-GCCACAAGTTCAGCGTGTCC and 5′-GCTTCTCGTTGGGGTCTTTC), HIF-1α (sima) (5′-CTGCGGGACTATCATA-

* This work was supported by an American Cancer Society Research Scholar grant (to E. A. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[2] The abbreviations used are: HIF-1, hypoxia-inducible factor-1; Hsp, heat shock protein; Hsf, heat shock factor; RNAi, RNA interference; GFP, green fluorescent protein; dsRNA, double-stranded RNA; HRE, hypoxia response element; RT-PCR, reverse transcription PCR.

ACAACC and 5′-AGGCTCAAAATCAATCTTTTGG), alternate HIF-1α (5′-GCATCACATCAAAGAGTCCCGAG and 5′-TCCGCAACCGTAACACCACTAC), and Hsf (5′-TGCCAAACAGTCCGCCTTATTAC and 5′-TGCTTTCCAAGTGCCCGTG). The T7 promoter sequence (5′-TAATACGACTCACTATAGGGAGA) was added 5′ to all above primers when ordered (IDT).

Reverse Transcription PCR—Total RNA was isolated using standard TRIzol protocols. Instructions from the Superscript III One-Step RT-PCR System with Platinum *Taq* (Invitrogen) were followed using 1 μg of total RNA, and 21 cycles of amplification were used for each test. The following primer pairs were used: HIF-1α (5′-CGAACTCGGTACTAAAGAACCTGC and 5′-GGGTCCTACTTTCACGCAAGG), Hsf (5′-ATCTGCTGCGTGGCGATG and 5′-CGTCCGTGTCCAAAATGTCG), Hsp26 (5′-ATGGCGTGCTCACCGTCAGTATTC and 5′-GGATGATGTTGGATGATGATGGCTC), Hsp27 (5′-AGGAGGAAGAAGACGACGAGATTCG and 5′-CATTGGGTGTGTTGTGGTGTGTCC), Hsp68 (5′-TTCACCACCTATGCCGACAACCAG and 5′-TCACATTCAGGATACCGTTTGCGTC), Hsp70Ab (5′-TCCATTCAGGTGTATGAGGGCG and 5′-CGTTCAGGATTCCATTGGCGTC), Hsp70Ba (5′-ACGATGCCAAGATGGACAAGGG and 5′-CGTCTGGGTTGATGGATAGGTTGAG), and Actin5c (5′-GGATGGTCTTGATTCTGCTGG and 5′-AGGTGGTTCCGCTCTTTTC).

Real-time PCR—Total RNA was isolated using standard TRIzol protocols. cDNA was synthesized following the SuperScript III Reverse Transcriptase protocol (Invitrogen). Real-time PCR was performed using the Sybr Green PCR Master Mix (Applied Biosystems) and an ABI PRISM 7900HT detection system (Applied Biosystems). The supplied analysis software was used for data interpretation. The following primer pairs were used: Hsf (5′-ACACCGCAGCCTCACATTATGACC and 5′-ATTTCCCTGGAGCAGCAAGTCCTC), Hsp27 (5′-AGGAGGAAGAAGACGACGAGATTCG and 5′-CATTGGGTGTGTTGTGGTGTGTCC), Hsp68 (5′-TTCACCACCTATGCCGACAACCAG and 5′-TCACATTCAGGATACCGTTTGCGTC), Hsp70Ab (5′-TCCATTCAGGTGTATGAGGGCG and 5′-CGTTCAGGATTCCATTGGCGTC), Hsp70Ba (5′-ACGATGCCAAGATGGACAAGGG and 5′-CGTCTGGGTTGATGGATAGGTTGAG), and Actin5c (5′-TGCTGGAGGAGGAGGAGGAGAAGTC and 5′-GCAGGTGGTTCGCTCTTTTCATC).

Hypoxia Reporter Construction—A small region of the *Hsf* intron containing the possible hypoxia regulatory elements was cloned into the Green H Pelican reporter vector. Kc_{167} cells were transfected using the Effectene kit (Qiagen) with this reporter and put in normoxia, hypoxia, or hypoxia with HIF-1α RNAi. Images were taken using a Nikon Eclipse TE2000-U microscope and MetaVue image capture software.

Chromatin Immunoprecipitation—Kc_{167} cells were transfected using the Effectene kit (Qiagen) with a pAc5.1/Sima plasmid that contains the full-length HIF-1α (sima) cDNA sequence with a c-terminal V5 epitope tag under the control of the Actin5c promoter. An equal quantity of mock transfected cells was used as a control, and all purification steps were carried out in parallel with the control and experimental cells. 24 h after transfection, control and experimental cells were incubated in hypoxia at room temperature for 24 h. DNA isolation and purification procedures followed standard V5 protocol.

PCR was used to detect *Hsf*, *Hph*, and *Actin5c* genomic regions in each of the samples of isolated DNA. 35 cycles were used to amplify 2 μl of template from each sample using the following primers: Hsf (5′-CTCCCACCACATACCGCTAATC and 5′-AAAAGCCAACTGAATGACCAAGG), Hph (5′-CCTTCTCACACTCCCTTCGCTG and 5′-CACTCTCTGCCAAGCCAAACC), Actin5c (5′-TGTGTGTGAGAGAGCGAAAGCC and 5′-CTGGAATAAACCGACTGAAAGTGG).

Larval Survival—First instar larvae of wild-type or flies with only one copy of the *Hsf* gene were counted and placed 10/vial of food. These vials were split and placed into groups for normoxic or hypoxia and reoxygenation stresses. This experimental group was maintained at 0.5% oxygen for 23 h and then placed in ambient oxygen for 1 h before returning to hypoxia. This cycle was repeated daily, and after 2 weeks the vials were scored for survival.

RESULTS

Hsf Transcript Levels Increase during Hypoxia in a HIF-1α-dependent Manner—D. melanogaster Kc_{167} cells were treated with GFP control double-stranded RNA (dsRNA) or dsRNA directed to eliminate transcripts of the *Drosophila* HIF-1α homologue, *similar* (20, 21), through RNAi. After exposure of the treated cells to normoxic or hypoxic conditions, total RNA was isolated for semi-quantitative reverse transcription PCR in order to characterize the transcript levels of HIF-1α, Hsf, and Actin5c as a control (Fig. 1*A*). Interestingly, we found that transcript levels of Hsf increased during hypoxia and that this upregulation was HIF-1α dependent. Cells lacking HIF-1α due to RNAi did not display a hypoxic increase in Hsf, instead maintaining Hsf levels more similar to control normoxic cells. Real-time PCR was then used to more accurately characterize these results (Fig. 1*B*) and further corroborated that Hsf transcripts increase under hypoxic conditions in a HIF-1α-dependent manner. As an additional control we repeated the RNAi with an alternate dsRNA sequence targeting another area of HIF-1α, which also showed a HIF-1α-dependent hypoxic increase of Hsf (Fig. 1*C*). This control experiment confirms our results were not due to off-target effects of the original RNAi.

The Increase in Hsf Transcript Levels during Hypoxia Is Directly Regulated by HIF-1α—The DNA recognition element to which HIF-1 binds during hypoxia contains a core 5′-RCGTG sequence (22). We had identified multiple instances of a related motif, 5′-TACGTGC, in the intron of the known HIF-1 target gene (23) HIF-1 prolyl hydroxylase and searched for this motif in the *Hsf* gene region. We identified two of these putative hypoxia response elements (HREs) in close proximity to one another in the second intron of *Hsf*. The two sites were 923 and 992 base pairs downstream from the transcriptional start site of *Hsf*, respectively. When this genomic region was aligned (24) with seven other *Drosophila* species these potential HREs were perfectly conserved (Fig. 2*A*). The sequence conservation of the two HRE motifs strongly suggests that there is evolutionary pressure to maintain these specific sequences.

We next tested whether these conserved HRE motifs had a regulatory function during hypoxia. A portion of the second

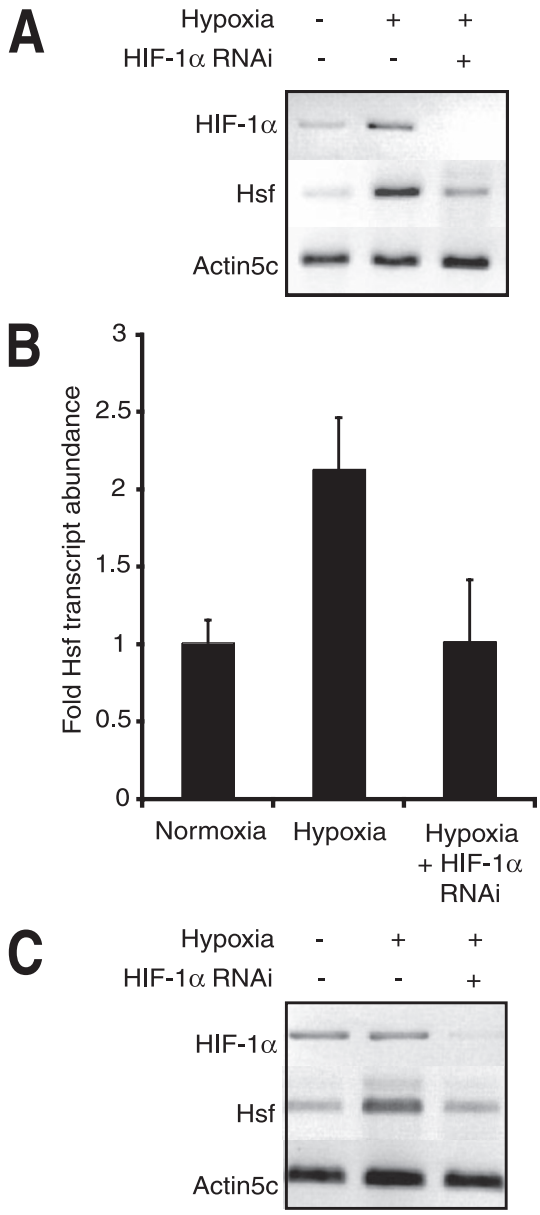

FIGURE 1. **Hsf transcript levels are increased in a HIF-1α-dependent manner.** *A*, RT-PCR analysis of the abundance of transcripts encoding HIF-1α, Hsf, and Actin5c (control) during normoxia or hypoxia in Kc_{167} cells. *Hsf* is up-regulated after hypoxia, and RNAi inactivation of HIF-1α eliminates this up-regulation. *B*, real-time PCR experiments confirm that RNAi inactivation of HIF-1α reduces the up-regulation of *Hsf* after hypoxia. S.E. of the mean is shown. Transcript changes in each condition are significantly different ($p < 0.05$). *C*, RT-PCR analysis of the abundance of transcripts during normoxia or hypoxia. An alternate dsRNA sequence targeting HIF-1α for RNAi showed similar results as in *panel A*, reducing the possibility that results from the original RNAi were due to nonspecific effects.

intron of *Hsf* containing the potential HREs was cloned upstream of a minimal promoter driving GFP in the Green H Pelican reporter vector (25). This reporter construct was then transfected into the Kc_{167} cell line and put under normoxic or hypoxic conditions. The hypoxic cells showed a dramatic increase in GFP fluorescence compared with the normoxic cells (Fig. 2*B*). The hypoxic increase in GFP expression was eliminated by HIF-1α RNAi treatment. The original reporter vector lacking the *Hsf* intron showed no hypoxic activation of GFP (data not shown), confirming that it was the cloned intronic region of *Hsf* that was leading to the HIF-1-dependent induction of the reporter during hypoxia.

The HRE-containing region was also tested by chromatin immunoprecipitation for direct binding by HIF-1. We transfected Kc_{167} cells with an epitope-tagged HIF-1α expression vector (26). DNA bound to HIF-1 protein during hypoxia was immunoprecipitated, and the *Hsf* intron genomic region as well as control genomic regions were PCR amplified to test for enrichment compared with DNA immunoprecipitated from a mock transfection. We found distinct enrichment of a 260-bp fragment encompassing the two HREs of the *Hsf* intron (Fig. 2*C*). As a positive control we showed an enrichment of a genomic region containing an HRE within the intron of the known HIF-1 target *Hph*. A negative control fragment located in an *Actin5c* intron showed no enrichment between the HIF-1α pulldown and the untransfected pulldown. These data indicate that *Hsf* is a direct target of HIF-1α through the binding of an intronic region containing two HREs that act as an enhancer of transcription during hypoxia.

Full Induction of Hsps during Hypoxia Is Dependent on HIF-1α Regulation of Hsf—The functional impact of the up-regulation of *Hsf* by HIF-1α on Hsp induction during hypoxia was then assayed. Kc_{167} cells were exposed to normoxia and hypoxia after treatment with control and HIF-1α RNAi, and reverse transcription PCR assayed transcript levels of various Hsps. All *Hsps* examined were dramatically up-regulated under hypoxia, and this increase was partly HIF-1α dependent (Fig. 3*A*). Hsp transcripts were not completely eliminated in hypoxic cells treated with HIF-1α dsRNA, presumably because the hypoxic stress activated the basal (normoxic) levels of Hsf protein already present in the cells. No HREs were found near any of the *Hsp* genes; therefore it is unlikely that HIF-1 was directly up-regulating these genes during hypoxia.

We tested whether the up-regulation of *Hsps* during hypoxia was dependent on Hsf. Cells were treated with control or Hsf RNAi and placed in normoxic and hypoxic conditions. When Hsf was removed through RNAi, Hsp transcripts were eliminated completely compared with the strong induction seen in cells treated with control dsRNA (Fig. 3*B*). Real-time PCR was used to more accurately quantify the results from both of the RNAi experiments. HIF-1α RNAi reduced the up-regulation of Hsps during hypoxia, yet Hsf RNAi completely removed Hsp transcripts (Fig. 3*C*). From these results, we can discern that Hsf regulates *Hsps*, while HIF-1 regulates *Hsf*.

The lack of strong *Hsp* up-regulation in hypoxic HIF-1 knockdown cells suggests that the HIF-1-mediated increase in Hsf transcript levels is an important step in regulating the sensitivity and activity of the heat shock response pathway. The functional impact of an increase in Hsf transcript levels in hypoxia was tested by assaying the response to hypoxia of a fly heterozygous for the null Hsf^1 mutation (27) and therefore containing only a single wild-type copy of *Hsf*. After exposure to hypoxia, these flies had reduced levels of Hsf transcripts compared with wild-type Oregon R flies as measured by real-time PCR (Fig. 4). The heterozygous flies with a reduction in Hsf transcripts also showed a strong reduction in Hsp26, Hsp27, and Hsp68 transcript levels compared with the control flies, although two *Hsp70* genes had normal levels of induction.

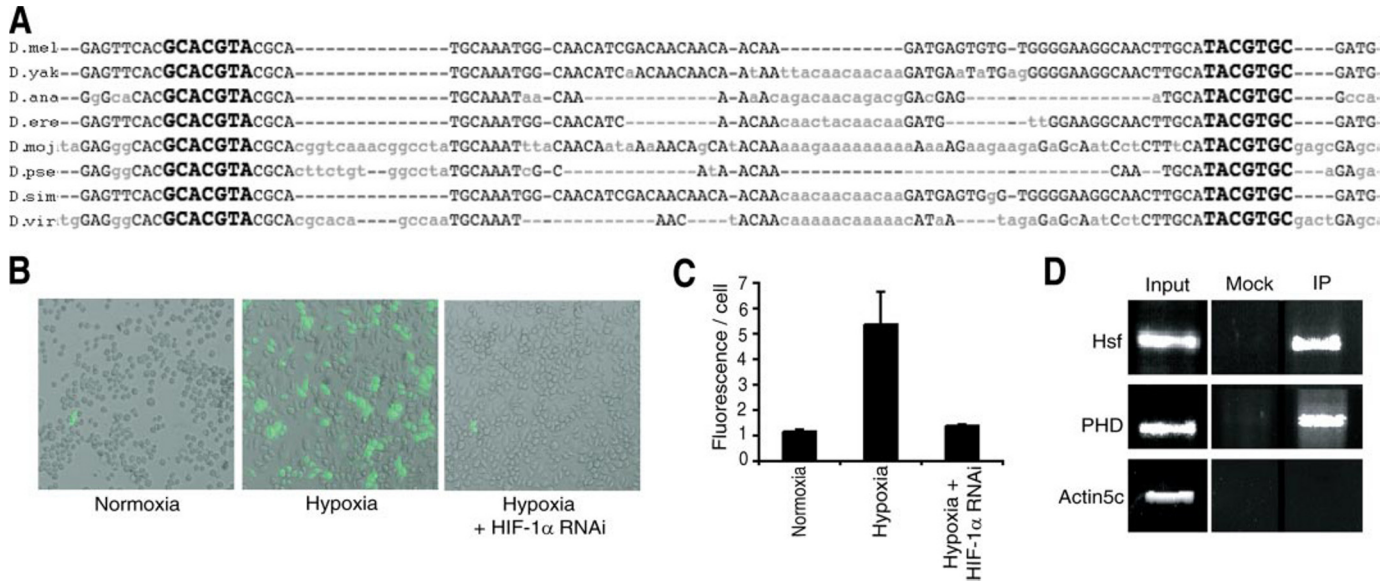

FIGURE 2. **The *Hsf* gene region has conserved HREs and is a direct target of HIF-1.** *A*, an alignment of the nucleotide sequence of the second intron of the *Hsf* gene in eight *Drosophila* species shows two HREs are fully conserved. *B*, the genomic sequence containing the two HREs in the *Hsf* intron was cloned up-stream of the minimal promoter of the Green H Pelican reporter vector. This construct was transfected into Kc_{167} cells and split into three conditions, normoxia, hypoxia, or hypoxia with HIF-1α RNAi. The reporter was not activated by normoxia, but hypoxia induced expression of the GFP reporter. The hypoxia activation of the reporter was eliminated by the addition of HIF-1α RNAi. *C*, fluorescence was measured using an ISS PC1 spectrofluorometer and normalized by cell number. Quantification confirmed a significant increase in fluorescence during hypoxia and a significant decrease from the hypoxic induction when HIF-1α RNAi was added to hypoxic cells. *D*, chromatin immunoprecipitation and PCR showing enrichment of the genomic region containing the two HREs within the *Hsf* gene in epitope-tagged HIF-1α-transfected *versus* untransfected Kc_{167} cells. The *Hph* gene and *Actin5c* genes were used as positive and negative controls, respectively.

These findings suggest that Hsf abundance impacts the up-regulation of some *Hsps* in a dose-dependent manner during hypoxia. Lower Hsf transcript abundance than the levels normally achieved during hypoxia are insufficient for the full up-regulation of *Hsps*.

Full Induction of Hsps and Viability during Reoxygenation Is Dependent on Increased Hsf Levels—During the return to normal oxygen conditions, Hsp levels remain high and are critical to tissue survival during this reoxygenation (28, 29). The effect of the HIF-1-dependent increase in Hsf level on Hsp expression persists during reoxygenation. Kc_{167} tissue culture cells with HIF-1α knocked down by RNAi had little increase in Hsp expression after hypoxia treatment and a reoxygenation period (Fig. 5*A*). Thus, the up-regulation of *Hsf* during hypoxia is critical to the high levels of Hsp transcripts during reoxygenation, as well as hypoxia.

Furthermore, we examined the functional importance *in vivo* of increased Hsf transcript abundance by assaying larval survival under hypoxia and reoxygenation stress. First instar larvae were reared in a regimen of alternating hypoxia and reoxygenation. The Hsf^1 heterozygotes had greatly reduced survival compared with larvae reared in normoxia (Fig. 5*B*). Control wild-type larvae showed no significant difference in survival between normoxia and the hypoxia and reoxygenation environments. These findings demonstrate the dosage importance of Hsf transcript levels for coping with hypoxia and reoxygenation at the organismal level.

Taken together, these experiments show the sequential order and importance of the hypoxia response. During hypoxia, HIF-1 directly up-regulates *Hsf*, which in turn up-regulates the whole family of *Hsps*. Without the HIF-1-regulated increase in Hsf, Hsps transcript levels never reach full induction during hypoxia or reoxygenation and organismal viability is reduced.

DISCUSSION

Up-regulation of *Hsps* during hypoxia is part of the canonical low oxygen stress response seen in *Drosophila* (30), *Caenorhabditis elegans* (13), and mammalian tissues (31). This study provides evidence that the up-regulation of *Hsf* during hypoxia surprisingly requires the activity of HIF-1, the effector of the low oxygen response. The transcriptional control of *Hsf* by HIF-1 has a functional impact on the activity of the heat shock response during hypoxia and the return to normal oxygen levels. Cells lacking HIF-1 or with reduced dosage of Hsf only increase Hsp transcript production slightly during low oxygen exposure and reoxygenation. The decreased production of Hsps reduces viability in flies experiencing hypoxia and reoxygenation, demonstrating that the full induction of the heat shock response is essential to counter the diverse physiological stresses associated with low oxygen.

Thus, we propose a model where HIF-1 directly up-regulates *Hsf* during hypoxia and the increased Hsf abundance in turn allows Hsf to further up-regulate *Hsps* during low oxygen exposure and also after the return to normal oxygen levels. The regulation of *Hsf* by HIF-1 provides a clear example of how cross-regulation between physiological stress response pathways can allow one pathway to sensitize the second and elicit a response under conditions where normally it would not be activated.

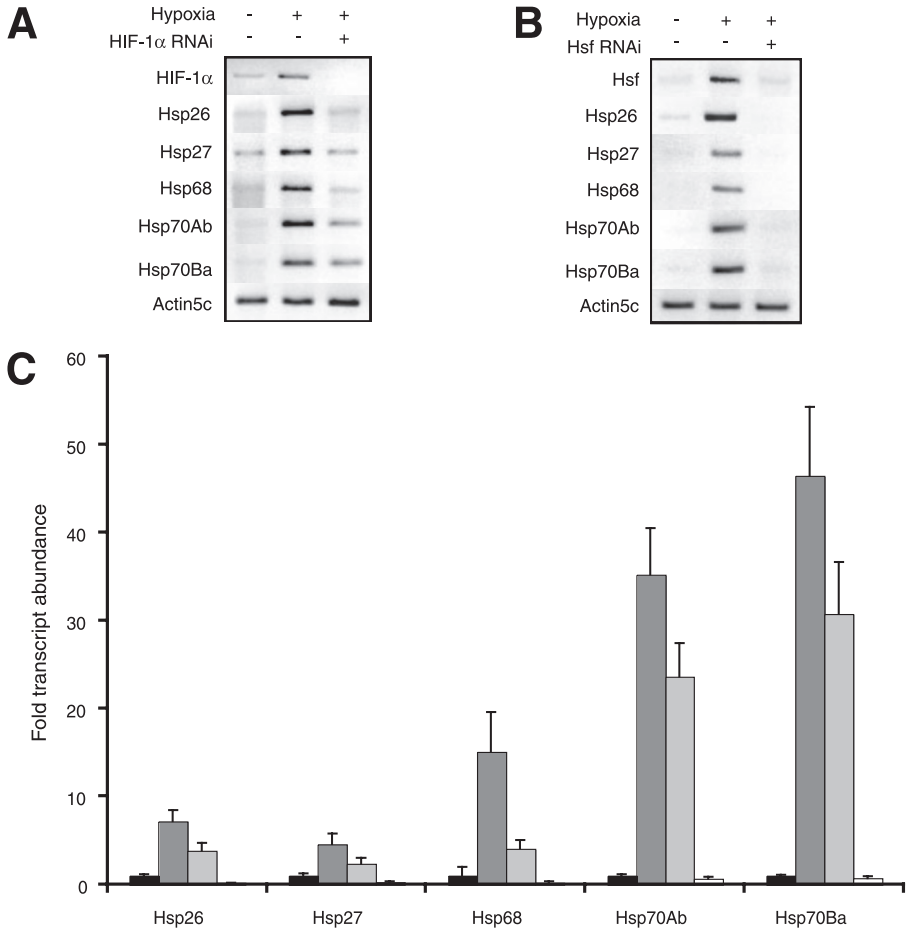

FIGURE 3. **Hsp transcript levels are increased in an Hsf- and HIF-1α-dependent manner during hypoxia.** *A*, RT-PCR of various transcripts involved in the heat shock pathway are all up-regulated after hypoxia. Inactivation of HIF-1α by RNAi reduces the increase in Hsp transcript abundance. Transcript levels of Actin5c are used as a control. *B*, RNAi of Hsf eliminates up-regulation of *Hsps* completely during hypoxia. Transcript levels of Actin5c are used as a control. *C*, real-time PCR analysis of transcripts from normoxic cells (*black bars*), hypoxic cells (*dark gray bars*), hypoxic cells treated with HIF-1α RNAi (*light gray bars*), and hypoxic cells treated with Hsf RNAi (*white bars*). Transcript levels of each Hsp were normalized to its normoxic level. Both RNAi treatments significantly reduced the transcript levels of all Hsps compared with hypoxia alone ($p < 0.05$).

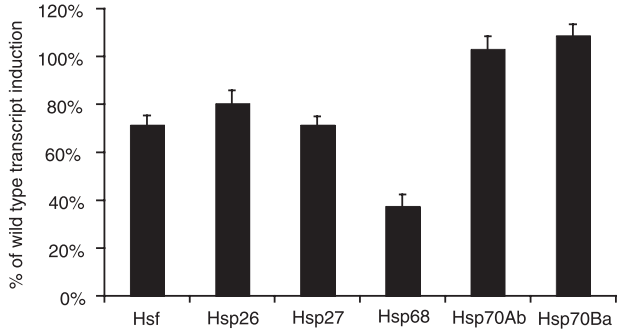

FIGURE 4. **Up-regulation of *Hsps* is Hsf dosage dependent.** Real-time PCR of flies heterozygous for a null *Hsf* mutation show a significant reduction in Hsf, Hsp26, Hsp27, and Hsp68 transcript abundance compared with wild-type flies after hypoxia ($p < 0.05$). This demonstrates that Hsf transcript abundance is critical to the magnitude of Hsp production. S.E. of the mean is shown.

Complex Regulation of Physiological Response Pathways—Cross-regulation between physiological pathways appears to be a feature of the low oxygen response. It has been shown that the insulin pathway can dramatically affect the HIF-1 pathway (32). Through the actions of the phosphatidylinositol 3-kinase/Akt pathway, HIF-1α translation is increased in a manner that outpaces the naturally normoxic degradation of HIF-1α (33). This leads to HIF-1 activation even when oxygen is present and up-regulating its downstream targets. Recently it has been shown that transforming growth factor-β1 activates the HIF-1 pathway by reducing the levels of prolyl hydroxylases that tag HIF-1α for degradation. Interestingly, it is also known that Hsp90 plays a role in stabilizing HIF-1α (34, 35). This mechanism is independent of the canonical oxygen-dependent regulation of HIF-1α and was the first evidence of any link between the heat shock and hypoxia stress pathways.

The cross-regulation between HIF-1 and Hsf found here is a new type of control, where the transcriptional effector of the low oxygen response directly regulates the transcript level of the effector of the heat shock response in order to sensitize the pathway. Interestingly, it has been already shown that HIF-1 and Hsf pathways have regulatory interactions, but in response to heat. Studies using *C. elegans* and rats showed that HIF-1 activity was essential for heat acclimation (36, 37). Our findings may explain the mechanism behind this phenomenon in that the increase in metabolic activity during high temperature may cause oxygen scarcity, thus stabilizing HIF-1 and increasing Hsf transcript levels.

Transcriptional Control of the Heat Shock Response—The activity of the heat shock pathway has been shown to be controlled by the trimerization and post-translational modification of Hsf protein subunits (18). Our results indicate that transcriptional control of *Hsf* is a means of further regulation of heat shock pathway activity. This transcriptional regulatory step is controlled by HIF-1, supporting a model in which the HIF-1 pathway causes increased *Hsf* transcription during hypoxia as a means to increase the cellular abundance of Hsf and increase the sensitivity of the heat shock pathway. In addition, the control of heat shock response sensitivity by HIF-1, the regulator of the low oxygen response, suggests that stress response pathways can assimilate complex new functions by regulating the transcriptional activators of other stress pathways.

Disease Implications—It has been shown that the increases in Hsp levels are critical for cell survival during hypoxia and the subsequent reoxygenation (29, 38). Our results indicate that it is through the HIF-1 pathway that the cell achieves this Hsp increase and is a means to protect against the stress of hypoxia. HIF-1 accumulation and activity have been linked to tumor

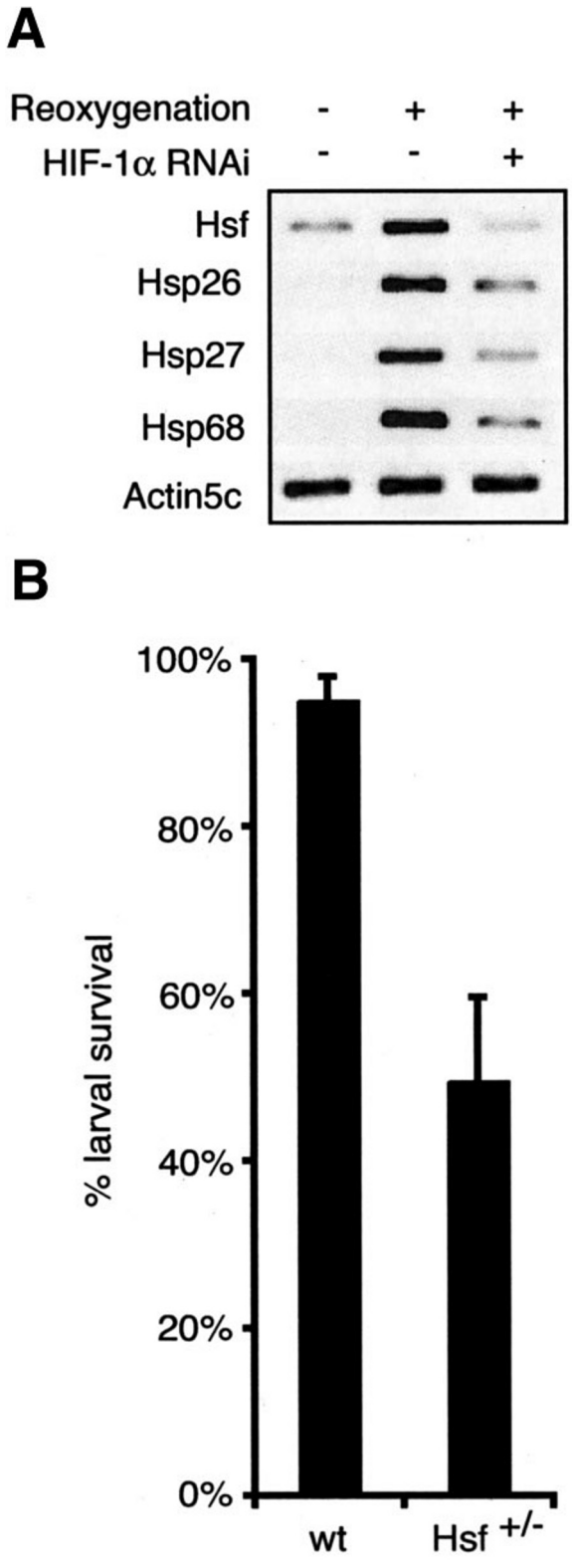

FIGURE 5. **Up-regulation of *Hsps* after reoxygenation is HIF-1α dependent and critical to survival.** *A*, RT-PCR of various transcripts involved in the heat shock pathway are all up-regulated after hypoxia. Inactivation of HIF-1α by RNAi reduces the increase in Hsp transcript abundance. Transcript levels of Actin5c are used as a control. *B*, larvae reared in either normoxia or hypoxia with a reoxygenation period each day were allowed to develop into pupae. Development of wild-type larvae was minimally affected by the hypoxic and reoxygenation stress. However, half as many $Hsf^{+/-}$ larvae reached the pupal stage when faced with repetitive hypoxia and reoxygenation compared with normoxic larvae. The reduction in survival was significant ($p < 0.05$). S.E. of the mean is shown.

progression, and various Hsps have also been shown to be crucial to cancer survival (39); thus, the hypoxic and heat shock response pathways play important roles in the pathophysiology of cancer. Our finding that the activity of HIF-1 controls the output of the heat shock pathway offers possible therapeutic approaches for mitigating hypoxic tissue damage and tumor growth by targeting this novel regulatory link.

Acknowledgments—We thank T. Gorr and H. Bunn for the V5-Sima expression vector and the Bloomington Stock Center for the fly lines.

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