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

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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), 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 Kc167 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% O2 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′-GCCACAGTTCAAGGGTTGCTCGGTCG-3′) and hypoxia response (HRE) (5′-CTGCGGGACTATCATCATAAGGGTTGCTCGGTCG-3′).

REFERENCES

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2. The abbreviations used are: HIF-1, hypoxia-inducible factor-1; Hsf, heat shock factor; Hsp, heat shock protein; dsRNA, double-stranded RNA; HRE, hypoxia response element; RT-PCR, reverse transcription PCR.
**HIF-1 Regulates Hsf Expression**

ACAACC and 5'-AGGCCTCAAAATCATACTTCTTTGG), alternate HIF-1α (5'-GCACTCAGCCATCGCGGAG and 5'-TCGGCAACCCGATAAAGGAC), and Hsf (5'-GTGCTGCAAGGACCGCATG and 5'-GTGTTTTGGAATGCGCG). The T7 promoter sequence (5'-TAATACGACTACA TAGGAGA) 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′-CGAAGTCTCTAGACTAAAGAATGCC and 5′-GGTGTCCTCTATTTTGAGCAG), Hsf (5′-ATCTGG- TGGTGGGCGGATG and 5′-GATTCCGTGCTGGTGCTTC), Hsp26 (5′-ATGGCCGTGCTCACTTGATATC), Hsp27 (5′-AGGAGGAAGACGAGGAGATCG and 5′-CTTGGGGTGATGGTTGTTGGTGC), Hsp70Ba (5′-TTCCACCCATATCGCCGCAACGAG and 5′-TCACATTCCGATACCGGTGCTG), Hsp70Ab (5′-TTCTGAGGATACCAGGTGAGG and 5′-GGTCTGGGTTGATGGGATGGTGG), and Actin5c (5′-AGGATGTTGTGGGATATGTTGGC and 5′-AGGATGTTGTGGCAGCCTTTC).

**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 Master Mix (Applied Biosystems) and an ABI PRISM 7900HT detection system (Applied Biosystems). The supplied analysis software was used to analyze the data. The following primer pairs were used: Hsf (5′-ACACGGCAGCCCTACATTATGACC and 5′-ATTTCCTTGAGGAGGAGATCTTCC), Hsp27 (5′-AGGAGGAGAAGACGAGGAGATC CG and 5′-CATTTGGGGTGGTTGTGTTGTTGC), Hsp68 (5′-TTCCACCCATATCGCCGCAACGAG and 5′-TCACATTCCGATACCGGTGCTG), Hsp70Ab (5′-TTCTGAGGATACCAGGTGAGG and 5′-GGTCTGGGTTGATGGGATGGTGG), and Actin5c (5′-AGGATGTTGTGGGATATGTTGGC and 5′-AGGATGTTGTGGCAGCCTTTC).

**RESULTS**

**Hsp Transcript Levels Increase during Hypoxia in a HIF-1α-dependent Manner**—D. melanogaster Kc167 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. 1A). Interestingly, we found that transcript levels of HIF increased during hypoxia and that this up-regulation was HIF-1α dependent. Cells lacking HIF-1α due to RNAi did not display a hypoxic increase in HIF, instead maintaining HIF levels more similar to control normoxic cells. Real-time PCR was then used to more accurately characterize these results (Fig. 1B) and further corroborated that HIF 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 HIF (Fig. 1C). 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. 2A). 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
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 Kc167 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. 2B). 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.

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. Kc167 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. 3A). 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. 3B). 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. 3C). 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 knockout 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 HsfΔ 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 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 Kc167 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.
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. Kc167 tissue culture cells with HIF-1/H9251 knocked down by RNAi had little increase in Hsp expression after hypoxia treatment and a reoxygenation period (Fig. 5A). 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 heterozygotes had greatly reduced survival compared with larvae reared in normoxia (Fig. 5B). 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.
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 thetranslational 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

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**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.
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.

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REFERENCES

1. Semenza, G. L. (1999) Annu. Rev. Cell Dev. Biol. 15, 551–578
2. Wang, G. L., Jiang, B. H., Rue, E. A., and Semenza, G. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5510–5514
3. Huang, L. E., Gu, J., Schau, M., and Bunn, H. F. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7987–7992
4. Ivan, M., Kondo, K., Yang, H., Kim, W., Vailiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S., and Kaelin, W. G., Jr. (2001) Science 292, 464–468
5. Jaakkola, P., Mole, D. R., Tian, Y. M., Wilson, M. I., Gielbert, J., Gaskell, S. J., Kriegsheim, A., Hebestreit, H. F., Mukherji, M., Schofield, C. J., Maxwell, P. H., Pugh, C. W., and Ratcliffe, P. J. (2001) Science 292, 468–472
6. Hon, W. C., Wilson, M. I., Harlos, K., Claridge, T. D., Schofield, C. J., Pugh, C. W., Maxwell, P. H., Ratcliffe, P. J., Stuart, D. I., and Jones, E. Y. (2002) Nature 417, 975–978
7. Huang, L. E., and Bunn, H. F. (2003) J. Biol. Chem. 278, 19575–19578
8. Pugh, C. W., and Ratcliffe, P. J. (2003) Nat. Med. 9, 677–684
9. Vogelstein, B., and Kinzler, K. W. (2004) Nat. Med. 10, 789–799
10. Semenza, G. L. (2002) Trends Mol. Med. 8, Suppl. 4, S62-S67
11. Semenza, G. L. (2003) Nat. Rev. Cancer 3, 721–732
12. Pousyssegur, J., Dayan, F., and Mazure, N. M. (2006) Nature 441, 437–443
13. Shen, C., Nettleton, D., Jiang, M., Kim, S. K., and Powell-Coffman, J. A. (2005) J. Biol. Chem. 280, 20580–20588
14. Wolch, W. J. (1993) Philos. Trans. R. Soc. Lond. B Biol. Sci. 339, 327–333
15. Westwood, J. T., Clos, J., and Wu, C. (1991) Nature 353, 822–827
16. Wu, C. (1995) Annu. Rev. Cell Dev. Biol. 11, 441–469
17. Pelham, H. R. (1982) Cell 30, 517–528
18. Orosz, A., Wisniewski, J., and Wu, C. (1996) Mol. Cell. Biol. 16, 7018–7030
19. Clemens, J. C., Worby, C. A., Simonson-Leff, N., Muda, M., Maehama, T., Hemmings, B. A., and Dixon, J. E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6499–6503
20. Nambu, I. R., Chen, W., Hu, S., and Crews, S. T. (1996) Gene 172, 249–254
21. Lavista-Llanos, S., Centanin, L., Irsenari, M., Russo, D. M., Gleadle, J. M., Bocca, S. N., Muzzopappa, M., Ratcliffe, P. J., and Wappner, P. (2002) Mol. Cell. Biol. 22, 6842–6853
22. Semenza, G. L., Jiang, B. H., Leung, S. W., Passantino, R., Concordet, J. P., Maire, P., and Giallongo, A. (1996) J. Biol. Chem. 271, 32529–32537
23. Aprikianova, O., Chandramouli, G. V., Wood, M., Vasselli, J. R., Riss, J., Maranchie, J. K., Linehan, W. M., and Barrett, J. C. (2004) J. Cell. Biochem. 92, 491–501
24. Bray, N., and Pachter, L. (2004) Genome Res. 14, 693–699
25. Barolo, S., Carver, L. A., and Posakony, J. W. (2000) BioTechniques 29, 726–732
26. Gorr, T. A., Tomita, T., Wappner, P., and Bunn, H. F. (2004) J. Biol. Chem. 279, 36048–36058
27. Jedlicka, P., Mordin, M. A., and Wu, C. (1997) EMBO J. 16, 2452–2462
28. Donnelly, T. J., Sievers, R. E., Vissern, F. L., Welch, W. J., and Wolfe, C. L. (1992) Circulation 85, 769–778
29. Kabakov, A. E., Budagova, K. R., Bryantsev, A. L., and Latchman, D. S. (2003) Cell Stress Chaperones 8, 335–347
30. Liu, G., Roy, J., and Johnson, E. A. (2006) Physiol. Genomics 25,
31. Morimoto, R. I. (1993) *Science* **259**, 1409–1410
32. Zelzer, E., Levy, Y., Kahana, C., Shilo, B. Z., Rubinstein, M., and Cohen, B. (1998) *EMBO J.* **17**, 5085–5094
33. Mottet, D., Dumont, V., Deccache, Y., Demazy, C., Ninane, N., Raes, M., and Michiels, C. (2003) *J. Biol. Chem.* **278**, 31277–31285
34. Minet, E., Mottet, D., Michel, G., Roland, I., Raes, M., Remacle, J., and Michiels, C. (1999) *FEBS Lett.* **460**, 251–256
35. Isaacs, J. S., Jung, Y. J., Mimnaugh, E. G., Martinez, A., Cuttitta, F., and Neckers, L. M. (2002) *J. Biol. Chem.* **277**, 29936–29944
36. Treinin, M., Shliar, I., Jiang, H., Powell-Coffman, J. A., Bromberg, Z., and Horowitz, M. (2003) *Physiol. Genomics* **14**, 17–24
37. Maloyan, A., Eli-Berchoer, L., Semenza, G. L., Gerstenblith, G., Stern, M. D., and Horowitz, M. (2005) *Physiol. Genomics* **23**, 79–88
38. Nakano, M., Mann, D. L., and Knowlton, A. A. (1997) *Circulation* **95**, 1523–1531
39. Ciocca, D. R., and Calderwood, S. K. (2005) *Cell Stress Chaperones* **10**, 86–103