HYPOXIA

mAKAP Compartmentalizes Oxygen-Dependent Control of HIF-1α

Wei Wong,[1] April S. Goehring,[1] Michael S. Kapiloff,[2] Lorene K. Langeberg,[3] John D. Scott[3]*

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The activity of the transcription factor hypoxia-inducible factor 1α (HIF-1α) is increased in response to reduced intracellular oxygen. Enzymes of the protein ubiquitin machinery that signal the destruction or stabilization of HIF-1α tightly control this transcriptional response. Here, we show that muscle A kinase–anchoring protein (mAKAP) organized ubiquitin E3 ligases that managed the stability of HIF-1α and optimally positioned it close to its site of action inside the nucleus. Functional experiments in cardiomyocytes showed that depletion of mAKAP or disruption of its targeting to the perinuclear region altered the stability of HIF-1α and transcriptional activation of genes associated with hypoxia. Thus, we propose that compartmentalization of oxygen-sensitive signaling components may influence the fidelity and magnitude of the hypoxic response.

INTRODUCTION

The concentration of cellular oxygen is maintained within a narrow range (termed normoxia) (*1*). A key cellular response to a state of reduced oxygen tension (hypoxia) involves induction of genes by the transcription factor known as hypoxia-inducible factor 1α (HIF-1α) (*2*). Under normoxic conditions, the abundance of HIF-1α is kept low through its ubiquitin-mediated proteasomal degradation (*3*). This process is initiated by the hydroxylation of two conserved proline residues, Pro^{402} and Pro^{564} in human HIF-1α, by a family of oxygen-sensitive dioxygenases called prolyl hydroxylases (PHD1, PHD2, and PHD3) (*4*, *5*). The hydroxylated proline residues constitute a binding site for the von Hippel–Lindau protein (pVHL), which is part of a complex that ubiquitinates HIF-1α and targets it for degradation by the proteasome (*6*).

During hypoxia, the continual destruction of HIF-1α is halted because of two factors: The enzymatic activities of the PHDs cease in the absence of oxygen, and seven in absentia homolog 2 (Siah2), a ubiquitin E3 ligase that contains a really interesting new gene (RING) domain, ubiquitinates select PHD isoforms and targets them for proteasomal degradation (*5*, *7*). Collectively, these mechanisms terminate the continual destruction of HIF-1α, which allows the protein to form a stable heterodimeric complex with the HIF-1β subunit (*8*–*11*). The HIF-1α–HIF-1β heterodimer accumulates in the nucleus and initiates transcription of proangiogenic, metabolic, and antiapoptotic genes that promote cell survival during hypoxia. These include *vascular endothelial growth factor* (*VEGF*), which encodes a protein that triggers the onset of angiogenesis to oxygen-deprived cells, and *glucose transporter 1* (*GLUT1*), which encodes a protein that enhances the uptake of glucose to stimulate glycolytic metabolism, even under aerobic conditions (*10*).

In the heart, accumulation of HIF-1α is an early marker of myocardial infarction (*12*). Although the molecular mechanisms underlying the destruction or maintenance of HIF-1α are well-defined (*2*), the subcellular organization of the factors that regulate these processes has not been investigated. Such compartmentalization could be achieved if regulatory factors of HIF-1α were assembled into multiprotein signaling complexes through their association with multivalent anchoring proteins. A kinase–anchoring proteins (AKAPs) are a growing family of functionally related scaffolding proteins that are frequently classified on the basis of their ability to sequester cyclic adenosine monophosphate (cAMP)–dependent protein kinase (PKA) in distinct subcellular compartments (*13*). However, selected members of the AKAP family also organize G protein–coupled receptors, GTPase (guanosine triphosphatase) effector proteins, and other classes of signaling enzymes (*14*–*18*). Hence, AKAPs are possible candidates to organize HIF-1α and its regulatory factors. Here, we show that muscle AKAP (mAKAP) organized components of the protein ubiquitin machinery that determined the fate of this transcription factor. During normoxia, mAKAP-mediated clustering of HIF-1α with negative regulatory factors enhanced its degradation. Under hypoxic conditions, however, positive regulatory factors in the mAKAP complex favored the stabilization of HIF-1α to initiate a transcriptional response.

RESULTS

AKAPs coordinate various signaling processes that affect aspects of acute and chronic cardiovascular pathophysiology (*19*–*21*). We therefore investigated several cardiac AKAPs as potential HIF-1α–interacting proteins. Human embryonic kidney (HEK) 293 cells were cotransfected with plasmids encoding V5 epitope–tagged HIF-1α (V5-HIF1α) and either green fluorescent protein (GFP)–tagged AKAP79, gravin, AKAP18α, or mAKAP. Western blots of immunoprecipitated complexes containing each AKAP were examined for coimmunoprecipitation of V5-HIF-1α with antibodies against GFP. Of these, only mAKAP coimmunoprecipitated with HIF-1α (Fig. 1A, top, lane 4). Reciprocal experiments detected mAKAP in V5-HIF-1α–containing complexes immunoprecipitated with an antibody against the V5 tag (Fig. 1B, top, lane 2). Control experiments established that mAKAP did not coimmunoprecipitate with another transcription factor, myocyte enhancer factor 2C (MEF2C) (Fig. 1B, top, lane 1). Detection of mAKAP in HIF-1α–containing complexes immunoprecipitated from the H9C2 rat cardiomyocyte–derived cell line showed

[1]Howard Hughes Medical Institute and Vollum Institute, Oregon Health & Science University, 3181 S. W. Sam Jackson Park Road, Portland, OR 97239, USA. [2]Departments of Medicine and Pediatrics, University of Miami, Miller School of Medicine, Miami, FL 33101, USA. [3]Howard Hughes Medical Institute and Department of Pharmacology, University of Washington School of Medicine, 1959 Pacific Avenue NE, HSB K-336B, Box 357370, Seattle, WA 98195, USA.
*To whom correspondence should be addressed. E-mail: scottjdw@u.washington.edu

an interaction between the endogenous proteins (Fig. 1C, top, lane 2). The extremely labile nature of HIF-1α under normoxic conditions (*2*, *22*) prevented isolation of such complexes from primary cultures of neonatal rat ventricular myocytes (NRVMs). Collectively, these studies suggested that mAKAP interacted with HIF-1α.

Ubiquitination of HIF-1α marks it for proteasomal degradation (*2*, *10*); thus, it seemed reasonable to establish whether it was ubiquitinated in the context of the mAKAP complex. To test this hypothesis, HEK 293 cells were transfected with plasmids encoding mAKAP and hemagglutinin (HA)-tagged ubiquitin. HA-ubiquitin was detected in mAKAP immu-

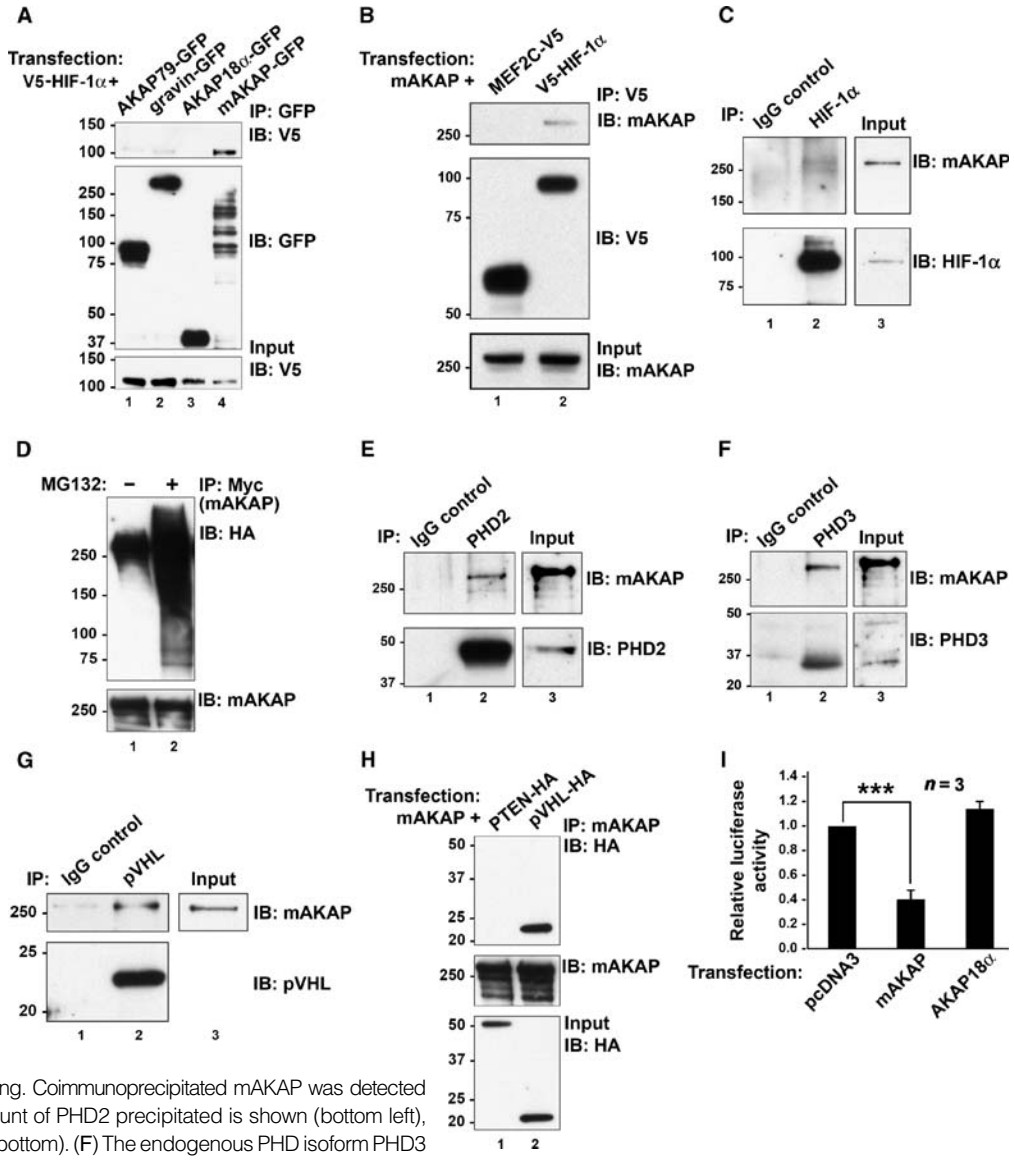

Fig. 1. mAKAP assembles components of the HIF-1α–degradation pathway. (**A**) GFP-tagged AKAPs (indicated) immunoprecipitated (IP) from HEK 293 cells were examined for the presence of V5-tagged HIF-1α by Western blotting (IB, top). AKAP-GFP–containing immunoprecipitates (middle) and V5-HIF-1α inputs (bottom) are shown. IP indicates the target that was immunoprecipitated; IB indicates the target that was analyzed by Western (immuno) blotting. (**B**) V5-tagged MEF2C and V5-HIF-1α were screened for their binding to mAKAP. Coimmunoprecipitation of mAKAP was detected by Western blotting (top). V5-tag–containing immunoprecipitates (middle), and the mAKAP inputs (bottom) are indicated. (**C**) Detection of the endogenous HIF-1α–mAKAP complex. HIF-1α was immunoprecipitated from H9C2 cells treated with the proteasome inhibitor MG132 and copurification of mAKAP (top) was detected by Western blotting. Input lanes for mAKAP (top) and HIF-1α (bottom) are indicated. IgG, immunoglobulin G. (**D**) The mAKAP complex immunoprecipitated from HEK 293 cells treated with MG132 or vehicle alone (indicated above each lane) was examined for ubiquitination. Incorporation of HA-tagged ubiquitin was detected by Western blotting (top), as was immunoprecipitated mAKAP (bottom). (**E**) The PHD isoform PHD2 was immunoprecipitated from NRVMs and screened for mAKAP binding. Coimmunoprecipitated mAKAP was detected by Western blotting (top left). The amount of PHD2 precipitated is shown (bottom left), as are the input proteins (right, top and bottom). (**F**) The endogenous PHD isoform PHD3 was immunoprecipitated from NRVMs and screened for mAKAP binding. Coimmunoprecipitated mAKAP was detected by Western blotting (top left). The amount of PHD3 precipitated is presented (bottom left) and protein inputs are also shown (right, top and bottom). (**G**) Endogenous pVHL was immunoprecipitated from NRVMs and screened for mAKAP binding. Coimmunoprecipitated mAKAP was detected by Western blotting (top left). The amount of immunoprecipitated pVHL is shown (bottom left). The amount of mAKAP in imput samples from both control and pVHL immunoprecipitates were detected by Western blotting (top right). (**H**) mAKAP immunoprecipitates from HEK 293 cells were tested for the coimmunoprecipitation of HA-tagged PTEN and pVHL by Western blotting (indicated above each lane, top). The amount of mAKAP in each immunoprecipitated sample is indicated (middle). PTEN and pVHL were detected in cell lysate input samples by Western blotting (bottom). Numbers to the left of panels indicate molecular weight markers (in kilodaltons). (**I**) HEK 293 cells were transfected with plasmids encoding mAKAP, AKAP18α, or vector alone. In addition, cells were transfected with both pVEGF, a firefly luciferase reporter vector for HIF-1α, and pTK-*Renilla*, a *Renilla* luciferase expression vector. Dual luciferase assays were performed on cell lysates and firefly luciferase values were normalized to *Renilla* values. Combined data from three independent experiments are shown. Statistical analysis of reporter data was done with one-way ANOVA followed by two-tailed Student's *t* test. Statistical significance of mAKAP versus pcDNA3 vector control was $P = 0.001$. Error bars represent SEM.

noprecipitates by Western blotting (Fig. 1D, top), and its abundance was enhanced on treatment with the proteasome inhibitor MG132 compared to that in immunoprecipitates from untreated cells (Fig. 1D, top, lane 2). This implies that HIF-1α was ubiquitinated when bound to mAKAP.

Proteasomal degradation of HIF-1α under normoxic conditions is triggered by hydroxylation of Pro^{402} and Pro^{564} (*4*). This posttranslational modification is catalyzed by the PHDs (*5*). The PHD2 and PHD3 isoforms are the principal means by which a low abundance of HIF-1α is maintained during normoxia in the heart (*23*, *24*). The substrate specificity of these enzymes is extremely restricted, with HIF-1α being their principal target (*24*, *25*). Hence, anchoring of PHDs in proximity to HIF-1α could ensure its rapid destruction under normoxic conditions. Accordingly, we detected mAKAP in PHD2- and PHD3-containing complexes immunoprecipitated from NRVM extracts (Fig. 1, E and F, top, lane 2). Control experiments indicated that PHD1 did not interact with mAKAP (fig. S1). Prolyl hydroxylation of HIF-1α mobilizes an E3 ubiquitin ligase complex containing the tumor suppressor protein pVHL (*6*); pVHL is also a component of the mAKAP complex. The anchoring protein was detected in pVHL-containing complexes immunoprecipitated from NRVM extracts (Fig. 1G, top, lane 2). Reciprocal studies showed the presence of pVHL in mAKAP immunoprecipitates from HEK 293 cells in which both proteins were expressed (Fig. 1H, top, lane 2). Control experiments confirmed that mAKAP did not bind nonspecifically to another HA-tagged protein, lipid phosphatase and tensin homolog deleted from chromosome 10 (PTEN) (Fig. 1H, top, lane 1, and fig. S2A).

The biochemical data presented thus far suggested that mAKAP assembled the enzymes that targeted HIF-1α for degradation. To test this notion by an alternative method, we examined whether mAKAP could suppress the transcriptional activity of HIF-1α in experiments with a luciferase reporter system driven by the promoter of the HIF-1α target *VEGF*. HIF-1α transcriptional activity was reduced by 59.6 ± 0.1% in mAKAP-transfected cells (Fig. 1I, column 2) compared to that in cells transfected with the control vector (Fig. 1I, column 1; $P = 0.001$). Control experiments showed that expression of another anchoring protein, AKAP18α, had no effect on the transcriptional activity of HIF-1α (Fig. 1I, column 3). This lends further support to the notion that mAKAP influences the degradation of HIF-1α under normoxic conditions.

The opposite is the case under hypoxic conditions in which HIF-1α accumulates because the activities of PHDs are inhibited (*22*). There are two aspects to this process: The reduced concentration of intracellular oxygen potently inhibits PHD activity, and another ubiquitin E3 ligase complex targets PHDs for proteosomal degradation (*5*, *7*). Siah2 is an essential component of the E3 ligase complex that catalyzes this latter step (*26*, *27*). Thus, we postulated that Siah2 and mAKAP might interact with each other. Accordingly, endogenous mAKAP was detected in complexes immunoprecipitated from NRVM extracts with an antibody against Siah2 (Fig. 2A, top, lane 2). Because Siah2 is itself ubiquitinated and thus becomes targeted for proteasomal degradation, our experiments were performed with a point mutant, Siah2 H99Y, which lacks a functional N-terminal RING domain and cannot promote its own degradation or that of the PHDs (*26*). Western blotting analysis of immunoprecipitated samples from transfected HEK 293 cells showed an interaction between mAKAP and Siah2 H99Y (Fig. 2B, top, lane 2), whereas control experiments confirmed that mAKAP did not interact with muscle-specific ring finger protein 1 (MuRF1), an E3 ubiquitin ligase that is structurally similar to Siah2 (Fig. 2B, top, lane 1, and fig. S2B).

If Siah2 inhibits PHD activity, then one could propose that the net effect of this inhibition would be stabilization of the mAKAP-associated pool of HIF-1α. Conversely, we postulated that incorporation of Siah2 H99Y into mAKAP-containing complexes would prevent suppression of PHD activity and ultimately enhance the destruction of HIF-1α. Experiments testing these predictions showed that HIF-1α was more abundant in mAKAP immunoprecipitates from cells expressing wild-type Siah2 (Fig. 2C, top, lane 1) than from cells expressing the H99Y mutant (Fig. 2C, top, lane 2). On the basis of our findings, we propose that mAKAP organizes a subcomplex of proteins that regulate the oxygen-dependent degradation and activation of HIF-1α. The proximity of Siah2 to the mAKAP-anchored pool of PHD increases the probability for ubiquitin-dependent degradation of PHD (Fig. 2D), which ultimately leads to the stabilization of HIF-1α (Fig. 2D). When we considered these results with our findings that mAKAP recruits enzymes that control the stability of HIF-1α (Fig. 1), it seemed reasonable to propose that this anchored signaling complex may augment the hypoxic transcriptional response.

Three complementary approaches were used to test this hypothesis in heart cells. RNA interference was used to knock down mAKAP in NRVMs (*28*). Depletion of the anchoring protein was confirmed by immunofluorescence microscopy (Fig. 3, A to F). NRVMs transfected with small interfering RNA (siRNA) specific for mAKAP displayed no immunore-

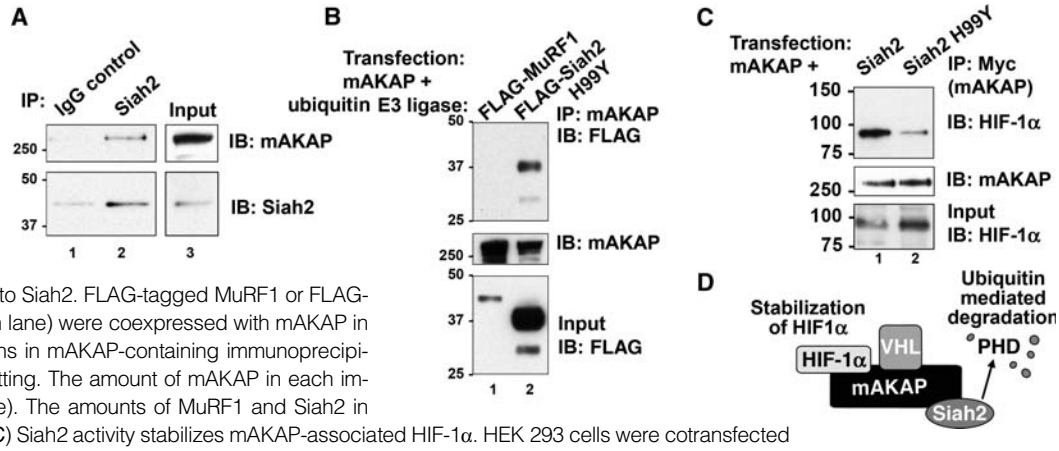

Fig. 2. mAKAP coordinates a HIF-1α–activation pathway. (**A**) Endogenous Siah2 binds to mAKAP in NRVMs. Siah2 was immunoprecipitated (IP) from NRVM lysates and copurification of mAKAP was detected by Western blotting (IB, top left). The amount of Siah2 immunoprecipitated (bottom) and mAKAP in NRVM lysates (top, right) are indicated. (**B**) mAKAP selectively binds to Siah2. FLAG-tagged MuRF1 or FLAG-tagged Siah2 (indicated above each lane) were coexpressed with mAKAP in HEK 293 cells. FLAG-tagged proteins in mAKAP-containing immunoprecipitates were detected by Western blotting. The amount of mAKAP in each immunoprecipitate is indicated (middle). The amounts of MuRF1 and Siah2 in cell lysates are indicated (bottom). (**C**) Siah2 activity stabilizes mAKAP-associated HIF-1α. HEK 293 cells were cotransfected with plasmids encoding mAKAP and either Siah2 or the Siah2 H99Y RING mutant. HIF-1α that coimmunoprecipitated with mAKAP was detected by Western blotting (top). The amount of mAKAP in each immunoprecipitate is indicated (bottom). Numbers to the left of panels indicate molecular weight markers (in kilodaltons). (**D**) Model showing how PHD becomes ubiquitinated by Siah2 in the mAKAP complex during hypoxia.

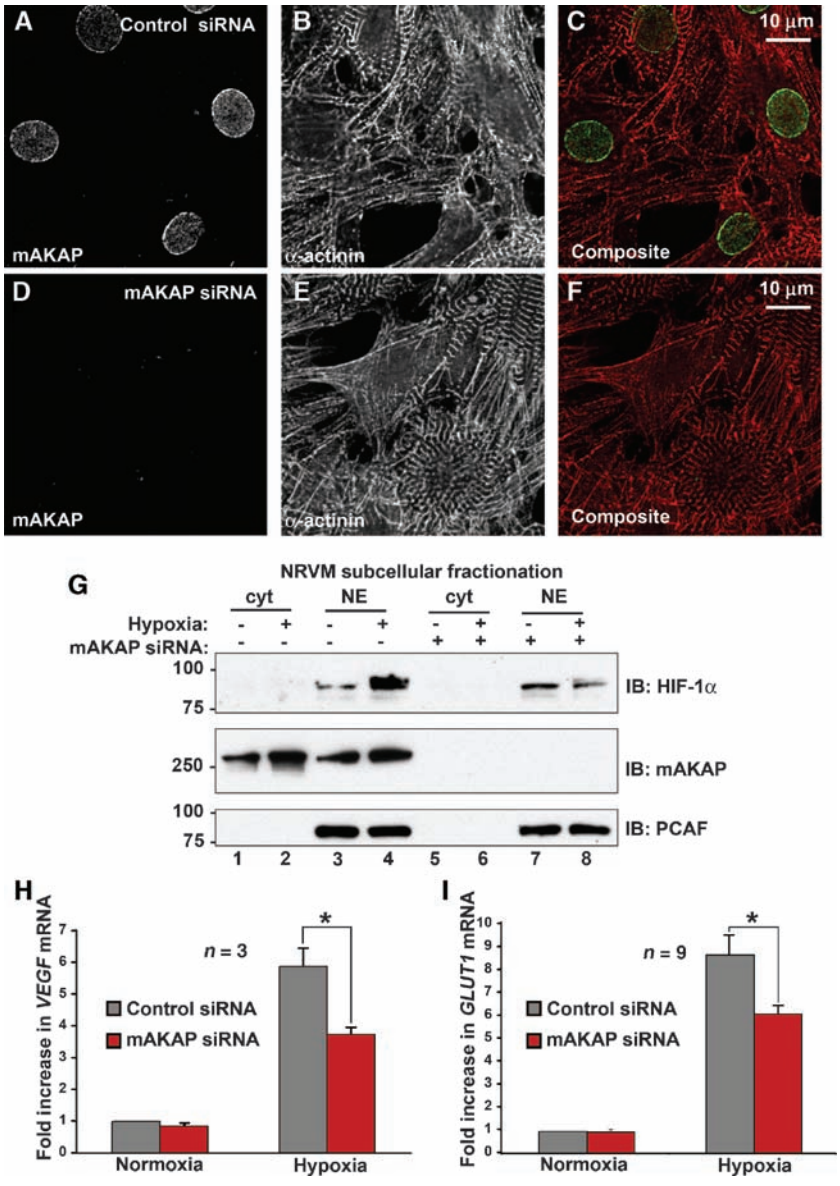

Fig. 3. siRNA-mediated knockdown of mAKAP reduces transcription of HIF-1α target genes in NRVMs. (**A** to **F**) NRVMs were transfected with control siRNA (A to C) or mAKAP-specific siRNA (D to F) and cultured for 72 hours. The cells were fixed and incubated with antibodies against mAKAP (A and D) and α-actinin (B and E), a marker for NRVM, and analyzed by immunofluorescence microscopy. Composite images (C and F) are also presented. (**G**) mAKAP-specific siRNA reduces the nuclear translocation of HIF-1α. NRVMs were transfected with control or mAKAP-specific siRNAs, exposed to normoxic or hypoxic conditions, and subjected to subcellular fractionation. Cytoplasmic (cyt) and nuclear (NE) fractions were analyzed by Western blotting for the presence of HIF-1α, mAKAP, and PCAF, a marker of the nuclear fraction. Numbers to the left of panels indicate molecular weight markers (in kilodaltons). (**H** and **I**) mAKAP-specific siRNA reduces the transcriptional activity of HIF-1α. NRVMs were transfected with control or mAKAP-specific siRNAs and exposed to normoxic or hypoxic conditions before RNA extraction. Quantitative real-time RT-PCR was performed to assess the expression of *VEGF* (H) and *GLUT1* (I) mRNAs, which were normalized to that of *18S rRNA*. Combined data from at least three independent experiments are shown. Statistical analysis of the RT-PCR data in (H) and (I) was done with one-way ANOVA followed by two-tailed Student's *t* test. Statistical significance of mAKAP versus control siRNA is $P = 0.03$ for *VEGF* and $P = 0.02$ for *GLUT1*. Error bars represent SEM.

activity to antibodies against mAKAP compared to that observed in their control siRNA-transfected counterparts (Fig. 3, A and D). Attempts to visualize endogenous HIF-1α by immunofluorescence in normoxic NRVMs were unsuccessful because of the rapid degradation of the newly synthesized protein. Therefore, changes in HIF-1α abundance were assessed by Western blotting analysis of NRVM extracts. HIF-1α was detected in the nuclear fractions of control NRVMs exposed to hypoxia (Fig. 3G, top, lane 4). Nuclear accumulation of HIF-1α was less pronounced in hypoxic NRVMs in which mAKAP had been knocked down by siRNA (Fig. 3G, top, lane 8). Western blotting analyses established that the abundance of mAKAP was reduced by the appropriate siRNA, and the integrity of the subcellular fractionation procedure was verified with an antibody against PCAF [P300- and cAMP response element–binding protein (CBP)–associated factor], a marker for the nuclear fraction (Fig. 3G, middle and bottom).

To confirm that the decrease in the nuclear translocation of HIF-1α altered the response to hypoxia, we performed quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assays to monitor the expression of two classic HIF-1α target genes, *VEGF* and *GLUT1* (*10*). VEGF and GLUT1 proteins are recognized as cellular markers for hypoxia (*29*, *30*). The abundance of *VEGF* messenger RNA (mRNA) was increased 5.9 ± 0.6-fold in hypoxic NRVMs compared to normoxic cells; so too was the abundance of *GLUT1* mRNA (8.6 ± 0.9-fold) (Fig. 3, H and I). However, knockdown of mAKAP by siRNA reduced the hypoxia-induced increase in *VEGF* and *GLUT1* expression by 33 and 25%, respectively, compared to that in control siRNA-transfected cells (Fig. 3, H and I). Thus, knockdown of mAKAP attenuated the HIF-1α–dependent response to hypoxia in NRVMs.

Further support for this concept was provided by the competitive displacement of the mAKAP complex from perinuclear membranes in NRVMs

(Fig. 4, A to F). This was achieved by adenoviral-mediated expression of the Myc-tagged mAKAP 340–1041 fragment, which competes with the native anchoring protein for its interaction with nesprin, an outer nuclear membrane protein (*31*). Accordingly, immunofluorescence studies showed that the distribution of endogenous mAKAP was cytoplasmic and diffuse upon expression of the 340–1041 fragment compared to that in control cells (Fig. 4, D to F). Subcellular fractionation experiments showed that mislocalization of mAKAP impeded the nuclear translocation of HIF-1α during hypoxia (Fig. 4G, top, lanes 4 and 8). Western blotting analysis confirmed that disruption of the targeting of mAKAP to the nucleus increased the amount of detectable anchoring protein in the cytoplasmic fraction (Fig. 4G, second panel, lanes 5 and 6). As before, detection of PCAF in the nuclear extracts confirmed the effectiveness of the fractionation procedure, and the presence of the Myc-tagged mAKAP 340–1041 fragment was also confirmed by Western blotting (Fig. 4G, third and bottom panels, lanes 5 and 6). The functional consequences of mislocalization of mAKAP were assessed by measuring changes in the expression of *VEGF* and *GLUT1* (Fig. 4, H and I). Under hypoxic conditions, the abundance of *VEGF* and *GLUT1* mRNAs was increased 7.3 ± 0.4-fold and 12.5 ± 1.0-fold, respectively, compared to that in normoxic cells (Fig. 4, H and I). However, competitive displacement of endogenous mAKAP by the mAKAP 340–1041 fragment blunted transcriptional activation of both hypoxia-associated genes by 24 and 28%, respectively, compared to that in control cells (Fig. 4, H and I). Therefore, we concluded that attachment of the

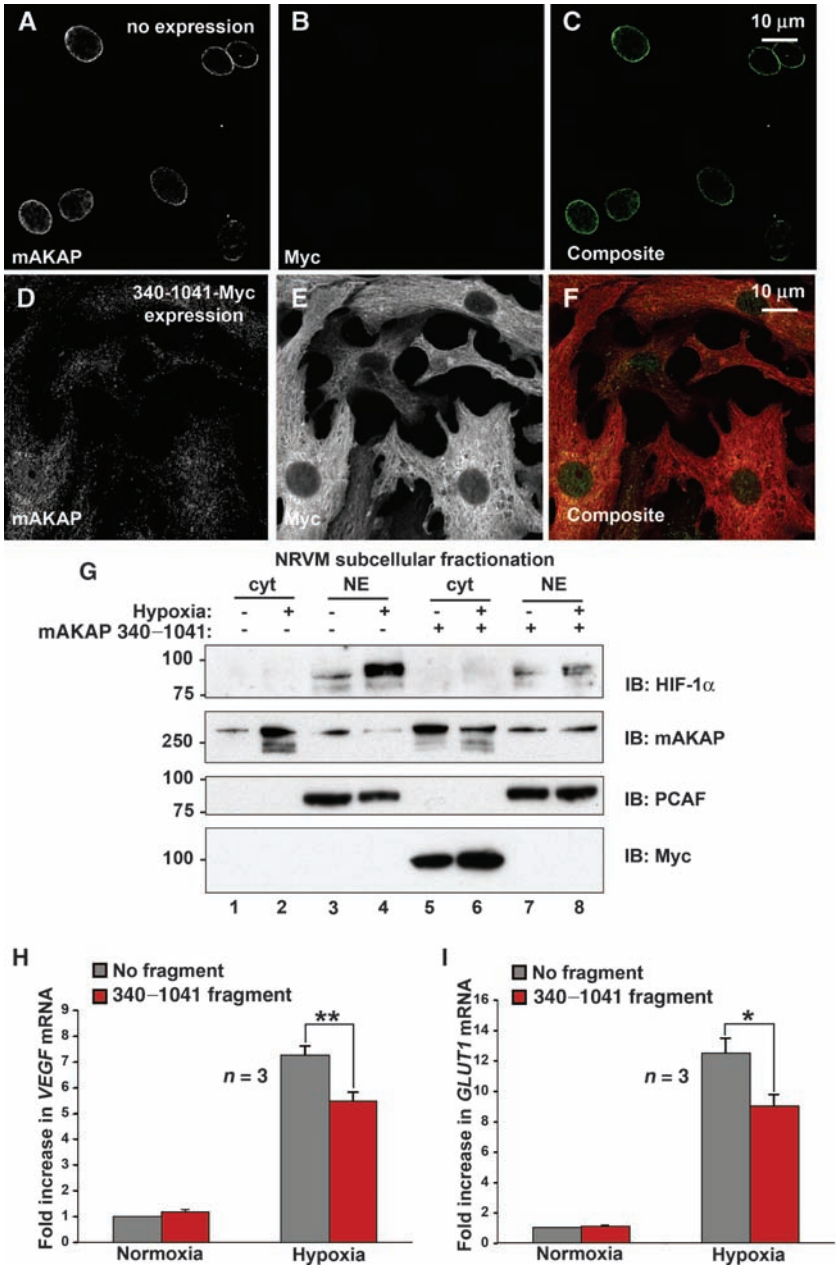

Fig. 4. Aberrant targeting of mAKAP reduces the expression of HIF-1α target genes in NRVMs. (**A** to **F**) NRVMs were infected with an adenovirus expressing the mAKAP 340–1041 fragment and a Tet-OFF adenovirus and cultured in doxycycline [50 ng/ml (A to C) or 0.5 ng/ml (D to F)] for 48 hours. The cells were fixed and incubated with antibodies against mAKAP (A and D) and Myc (B and E) and analyzed by immunofluorescence microscopy. Composite images (C and F) are also presented. (**G**) The mAKAP 340–1041 fragment reduces the nuclear translocation of HIF-1α. NRVMs were infected, exposed to normoxic or hypoxic conditions, and subjected to subcellular fractionation. Cytoplasmic (cyt) and nuclear (NE) fractions were analyzed by Western blotting for HIF-1α, mAKAP, PCAF, and Myc. Numbers to the left of panels indicate molecular weight markers (in kilodaltons). (**H** and **I**) The mAKAP 340–1041 fragment reduces the transcriptional activity of HIF-1α. NRVMs were infected and exposed to normoxic or hypoxic conditions before RNA extraction. Quantitative real-time RT-PCR was performed to assess the expression of *VEGF* (H) and *GLUT1* (I) mRNAs, which were normalized to that of *18S rRNA*. Combined data from three independent experiments are shown. Statistical analysis of these data was done with one-way ANOVA followed by two-tailed Student's *t* test. Statistical significance of mAKAP versus control siRNA is $P = 0.02$ for *VEGF* and $P = 0.05$ for *GLUT1*. Error bars represent SEM.

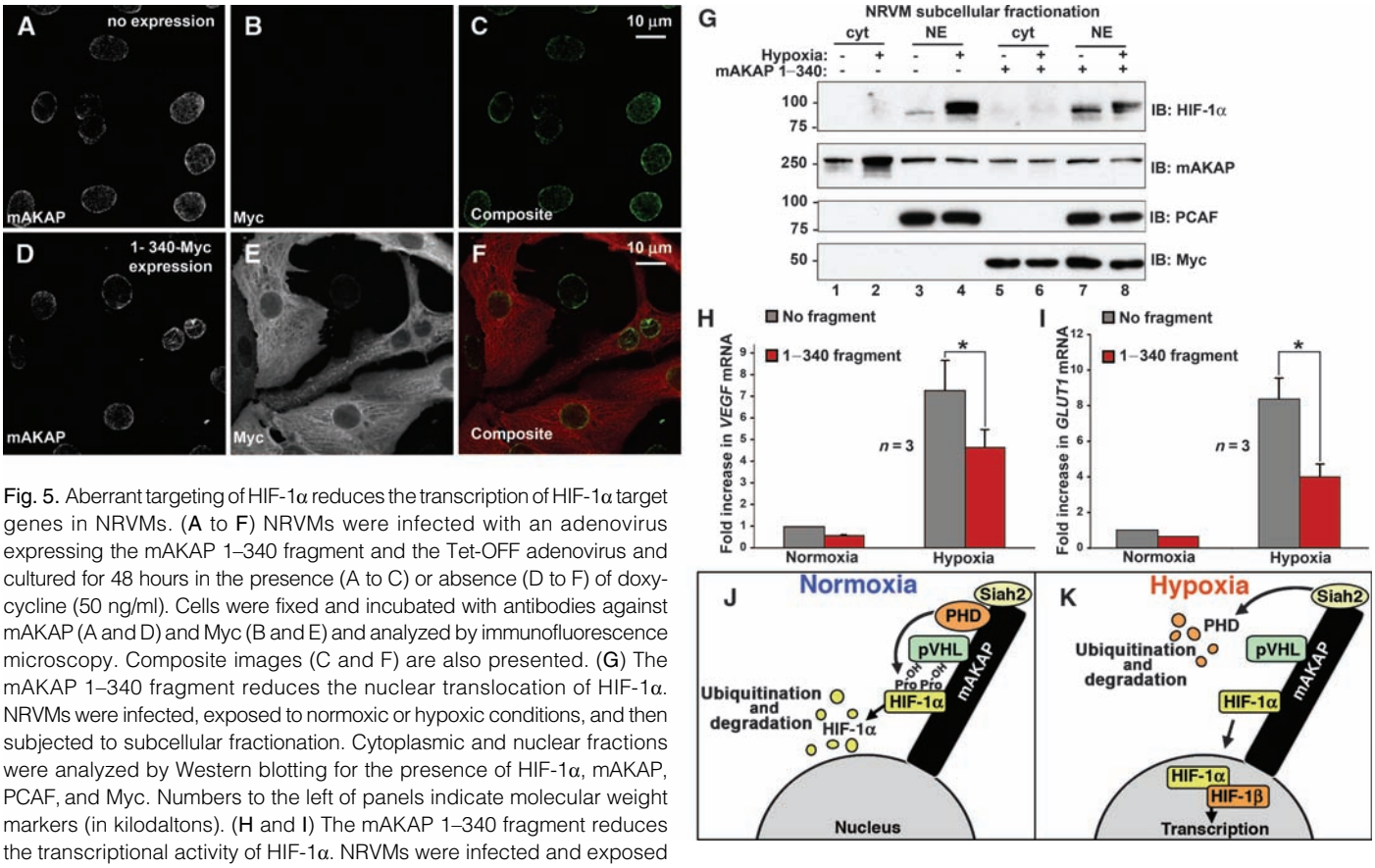

Fig. 5. Aberrant targeting of HIF-1α reduces the transcription of HIF-1α target genes in NRVMs. (**A** to **F**) NRVMs were infected with an adenovirus expressing the mAKAP 1–340 fragment and the Tet-OFF adenovirus and cultured for 48 hours in the presence (A to C) or absence (D to F) of doxycycline (50 ng/ml). Cells were fixed and incubated with antibodies against mAKAP (A and D) and Myc (B and E) and analyzed by immunofluorescence microscopy. Composite images (C and F) are also presented. (**G**) The mAKAP 1–340 fragment reduces the nuclear translocation of HIF-1α. NRVMs were infected, exposed to normoxic or hypoxic conditions, and then subjected to subcellular fractionation. Cytoplasmic and nuclear fractions were analyzed by Western blotting for the presence of HIF-1α, mAKAP, PCAF, and Myc. Numbers to the left of panels indicate molecular weight markers (in kilodaltons). (**H** and **I**) The mAKAP 1–340 fragment reduces the transcriptional activity of HIF-1α. NRVMs were infected and exposed to normoxic or hypoxic conditions before RNA extraction. Quantitative real-time RT-PCR was performed to assess the expression of *VEGF* (H) and *GLUT1* (I) mRNAs, which were normalized to that of *18S rRNA*. Combined data from three independent experiments are shown. Statistical analysis was done with one-way ANOVA followed by two-tailed Student's *t* test. Statistical significance of mAKAP versus control siRNA is $P = 0.04$ for *VEGF* and $P = 0.01$ for *GLUT1*. Error bars represent SEM. (**J** and **K**) Model showing the proposed role of mAKAP in the regulation of the degradation of HIF-1α during normoxia and in the regulation of its activation during hypoxia.

mAKAP complex to perinuclear membranes of myocytes influenced the HIF-1α–dependent hypoxic response.

Our final approach was to selectively displace HIF-1α and its regulatory enzymes from the mAKAP signaling complex and measure any resulting changes in the hypoxic response. Initial experiments mapped the HIF-1α–binding site of mAKAP to the first 340 amino acid residues of the anchoring protein (fig. S3, A and B, top). Parallel studies showed that pVHL and Siah2 bound to the same region of mAKAP (fig. S3B, second and third panels). This information was then used to generate an inducible adenoviral vector encoding this region of mAKAP (residues 1–340) that could displace HIF-1α and its regulatory enzymes from the mAKAP signaling complex in NRVMs. Immunofluorescence microscopy confirmed that this mAKAP fragment did not alter the subcellular distribution of the endogenous anchoring protein in NRVMs (Fig. 5, A to F). However, subcellular fractionation of NRVMs showed that the mAKAP 1–340 fragment reduced the nuclear accumulation of HIF-1α under hypoxic conditions (Fig. 5G, top, lanes 4 and 8) compared to that in cells not expressing the mAKAP 1–340 fragment. Furthermore, displacement of HIF-1α from its regulatory enzymes reduced the transcription of hypoxia-responsive genes. Expression of the mAKAP 1–340 fragment in NRVMs reduced the hypoxia-induced expression of *VEGF* and *GLUT1* mRNAs by 52 and 60%, respectively, when compared to that of controls (Fig. 5, H and I).

DISCUSSION

These findings suggest an unanticipated role for mAKAP in cellular oxygen homeostasis by functioning as a scaffolding protein that determines the fate of HIF-1α. During normoxia, mAKAP-mediated clustering of HIF-1α with regulatory factors such as PHDs and pVHL provided a means to enhance the efficiency of its ubiquitin-mediated degradation (Fig. 5J). Under hypoxic conditions, however, another E3 ligase, Siah2, favored the stabilization of HIF-1α (Fig. 5K). The expression of HIF-1α target genes protects against ischemic insult in organs vulnerable to injury from oxygen deprivation, such as the heart and the brain (*32*). We now propose that mAKAP, an anchoring protein that is found primarily in cardiomyocytes and neurons, is an additional regulatory element that enhances the hypoxic response.

Another intriguing implication is that this anchoring protein may organize components of the ubiquitination machinery to form a localized degradation loop for HIF-1α. Thus, mAKAP is one of a few anchoring proteins that interface with ubiquitin pathways. Interestingly, each anchoring protein does so in a different manner. For example, ubiq-

uitination of the AKAP79/150 binding partner PSD-95 regulates the abundance of ion channels at the cell surface during synaptic plasticity (*33*), whereas ubiquitin-mediated proteasomal degradation of the mitochondrial anchoring protein AKAP121 affects the oxidative capacity of these organelles (*34*). In contrast, mAKAP was not marked for proteasomal degradation but organized components of the ubiquitination machinery to facilitate the bidirectional control of the stability of HIF-1α (Fig. 2D).

Because it is tethered to the perinuclear membrane in cardiomyocytes and its abundance is increased under conditions of cardiac stress, mAKAP is a particularly attractive candidate to compartmentalize HIF-1α (*13*, *28*, *35*). Sequestering of HIF-1α at this location could minimize the translocation distance to its site of action in the nucleus. These findings tally with a fundamental tenet of the "anchoring hypothesis," namely, that AKAPs direct signaling enzymes toward preferred substrates (*13*, *36*). It has been our experience that these anchored enzymes can be restricted spatially (*37*–*40*). For example, we have recently shown that a 2- to 3-μm displacement of PKA in dendritic spines has profound effects on excitatory synaptic transmission in neurons isolated from AKAP150 knockout mice (*41*). Data presented here (Figs. 4 and 5) add to our understanding of this concept in various ways. We showed that displacement of mAKAP from the perinuclear membranes of cardiomyocytes repressed the hypoxic response. There are two notable features of the mAKAP-mediated translocation of HIF-1α. The anchoring protein not only organizes enzymes that affect the stability of HIF-1α, but also functions to optimally position the transcription factor close to its site of action inside the nucleus. Hence, previously unappreciated regulatory elements such as the perinuclear mAKAP complex that sequester stabilized HIF-1α close to its site of action may enhance a rapid transcriptional response. This could aid myocyte survival in an acute and adverse ischemic environment.

Components of the mAKAP complex could also assist in the accumulation of HIF-1α under pathophysiological conditions, in which oxygen supply to the heart is limited. This agrees with evidence that the abundance of HIF-1α is increased upon myocardial infarction and that increasing its abundance during pressure overload prevents the transition from cardiac hypertrophy to heart failure (*42*). These findings infer a link between hypoxia and hypertrophic signaling pathways. Indeed, mAKAP may provide such a link because the abundance of mAKAP is increased in response to hypertrophic stimuli (*35*). Further support for this notion comes from evidence that mAKAP anchors two signaling enzymes that act sequentially to influence cardiomyocyte hypertrophy and the stability of HIF-1α. One of these, the cAMP-responsive guanine nucleotide exchange factor Epac-1 (*28*), is the upstream element in a signaling pathway that modulates the activity of extracellular signal–regulated kinase 5 (ERK5), a protein kinase that augments the hypertrophic response (*43*) and influences the stability of HIF-1α (*44*). Thus, certain mAKAP complexes may create cellular microenvironments in which cAMP signals can feed into oxygen-responsive transcriptional activation pathways. This underscores an emerging aspect of the biology of AKAPs: that the multiprotein complexes that they maintain not only control second messenger signaling events but also shape broader aspects of cellular regulation.

MATERIALS AND METHODS

Antibodies

The following primary antibodies were used for immunoprecipitations and Western blotting: mouse monoclonal anti-FLAG (Sigma, St. Louis, MO), mouse monoclonal anti-HA (Sigma), rabbit polyclonal anti-GFP (Molecular Probes, Eugene, OR), mouse monoclonal anti–HIF-1α (Novus Biologicals, Littleton, CO), rabbit polyclonal anti-mAKAP (VO54 and Covance, Princeton, NJ), mouse monoclonal anti-mAKAP (Covance), mouse monoclonal anti-Myc (Santa Cruz Biotechnology, Santa Cruz, CA, and Upstate, Lake Placid, NJ), mouse monoclonal anti-PCAF (Santa Cruz Biotechnology), rabbit polyclonal anti-PHD2 (Novus Biologicals), rabbit polyclonal anti-PHD3 (Novus Biologicals), mouse monoclonal anti-V5 (Invitrogen, Carlsbad, CA), mouse monoclonal anti-Siah2 (Sigma), mouse monoclonal anti–α-tubulin (Sigma), rabbit polyclonal anti-pVHL (Santa Cruz Biotechnology), and mouse monoclonal anti-VSV (Sigma).

Plasmid constructs

To construct plasmids encoding HIF-1α, MEF2C, PHD1, Siah2, and MuRF1, clones from the Mammalian Gene Collection (Open Biosystems, Huntsville, AL) were used as templates for PCR. The QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) was used to introduce two point mutations (Pro^{402}→Ala^{402} and Pro^{564}→Gly^{564}) in the HIF-1α construct to abolish oxygen-dependent prolyl hydroxylation (*4*, *45*) and a single point mutation (His^{99}→Tyr^{99}) in Siah2 to create a nonfunctional RING domain (*46*).

Small interfering RNAs

The custom-synthesized mAKAP-specific siRNA (Dharmacon, Lafayette, CO) was based on our previously characterized short hairpin RNA (*28*). The HIF-1α–specific siRNA pool was obtained from Dharmacon and the negative control siRNA was obtained from Qiagen (Valencia, CA).

Cell culture, transfection, and infection

HEK 293 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were transfected with the appropriate plasmids with calcium phosphate or Lipofectamine Plus (Invitrogen). Primary NRVMs were prepared as previously described (*47*). NRVMs were transfected with control or mAKAP-specific siRNAs with DharmaFECT1 and were analyzed 72 hours later. Adenoviral infection of NRVMs was performed as previously described (*47*).

Immunocytochemistry, immunoprecipitations, and Western blotting

NRVMs were fixed, permeabilized, and incubated with primary and secondary antibodies as previously described (*35*). Immunoprecipitations and Western blotting analyses were performed as previously described (*48*).

Reporter gene assays

HEK 293 cells were plated at a density of 5×10^4 cells per well and transfected with pcDNA3 and plasmids encoding AKAP18α or mAKAP, as well as pVEGF (American Type Culture Collection, Manassas, VA) and pTK-*Renilla* (a gift from R. H. Goodman) with *Trans*IT-LT1 (Mirus Bio, Madison, WI). Cells were lysed in Passive Lysis Buffer (Promega, Madison, WI), and the ratio of firefly to *Renilla* luciferase activity was determined with the Dual-Luciferase Reporter Assay System (Promega).

Ubiquitination reactions

HEK 293 cells were treated with 40 μM MG132 (Calbiochem, Gibbstown, NJ) or vehicle (dimethyl sulfoxide) for 4 hours at 37°C. Immunoprecipitations were performed as described above.

Hypoxic treatment

NRVM medium was incubated overnight in a modular incubator chamber (Billups-Rothenberg, Del Mar, CA) flushed with the appropriate gas mix: 5% CO_2 and 95% N_2 for experiments to analyze HIF-1α protein, or 1% O_2, 5% CO_2, and 95% N_2 for experiments to analyze HIF-1α transcriptional activity. The equilibrated medium was added to cells, which were transferred to the modular incubator chamber. The chamber was flushed again, and cells were incubated at 37°C for 4 hours for protein analysis and 6 hours for transcriptional analysis.

Subcellular fractionation

NRVMs were separated into cytosolic fractions and nuclear extracts with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL) according to the manufacturer's instructions.

Real-time RT-PCR

Total RNA was extracted from NRVMs with the RNAeasy (Qiagen) or Absolutely RNA (Stratagene) miniprep kits. RNA was converted to complementary DNA (cDNA) with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The abundance of *VEGF* and *GLUT1* mRNAs was assessed with TaqMan Gene Expression Assays and normalized to that of *18S ribosomal RNA* (*rRNA*).

Statistical analysis

All statistical tests were carried out with one-way analysis of variance (ANOVA), followed by two-tailed unpaired Student's *t* tests with Origin (OriginLab Corporation, Northampton, MA). All alpha levels were set to 0.05.

SUPPLEMENTARY MATERIALS

www.sciencesignaling.org/cgi/content/full/1/51/ra18/DC1
Fig. S1. PHD1 does not interact with mAKAP.
Fig. S2. pVHL and Siah2 bind to mAKAP.
Fig. S3. Mapping of mAKAP-binding regions.

REFERENCES AND NOTES

1. F. J. Giordano, Oxygen, oxidative stress, hypoxia, and heart failure. *J. Clin. Invest.* **115**, 500–508 (2005).
2. G. L. Semenza, HIF-1 and mechanisms of hypoxia sensing. *Curr. Opin. Cell Biol.* **13**, 167–171 (2001).
3. M. Ohh, C. W. Park, M. Ivan, M. A. Hoffman, T. Y. Kim, L. E. Huang, N. Pavletich, V. Chau, W. G. Kaelin, Ubiquitination of hypoxia-inducible factor requires direct binding to the β-domain of the von Hippel–Lindau protein. *Nat. Cell Biol.* **2**, 423–427 (2000).
4. P. Jaakkola, D. R. Mole, Y. M. Tian, M. I. Wilson, J. Gielbert, S. J. Gaskell, A. von Kriegsheim, H. F. Hebestreit, M. Mukherji, C. J. Schofield, P. H. Maxwell, C. W. Pugh, P. J. Ratcliffe, Targeting of HIF-α to the von Hippel–Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* **292**, 468–472 (2001).
5. A. C. Epstein, J. M. Gleadle, L. A. McNeill, K. S. Hewitson, J. O'Rourke, D. R. Mole, M. Mukherji, E. Metzen, M. I. Wilson, A. Dhanda, Y. M. Tian, N. Masson, D. L. Hamilton, P. Jaakkola, R. Barstead, J. Hodgkin, P. H. Maxwell, C. W. Pugh, C. J. Schofield, P. J. Ratcliffe, *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107**, 43–54 (2001).
6. P. H. Maxwell, M. S. Wiesener, G. W. Chang, S. C. Clifford, E. C. Vaux, M. E. Cockman, C. C. Wykoff, C. W. Pugh, E. R. Maher, P. J. Ratcliffe, The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271–275 (1999).
7. K. Nakayama, I. J. Frew, M. Hagensen, M. Skals, H. Habelhah, A. Bhoumik, T. Kadoya, H. Erdjument-Bromage, P. Tempst, P. B. Frappell, D. D. Bowtell, Z. Ronai, Siah2 regulates stability of prolyl-hydroxylases, controls HIF1α abundance, and modulates physiological responses to hypoxia. *Cell* **117**, 941–952 (2004).
8. G. L. Wang, B. H. Jiang, E. A. Rue, G. L. Semenza, Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5510–5514 (1995).
9. G. L. Wang, G. L. Semenza, Purification and characterization of hypoxia-inducible factor 1. *J. Biol. Chem.* **270**, 1230–1237 (1995).
10. M. Y. Koh, T. R. Spivak-Kroizman, G. Powis, HIF-1 regulation: Not so easy come, easy go. *Trends Biochem. Sci.* **33**, 526–534 (2008).
11. R. H. Wenger, M. Gassmann, Oxygen(es) and the hypoxia-inducible factor–1. *Biol. Chem.* **378**, 609–616 (1997).
12. S. H. Lee, P. L. Wolf, R. Escudero, R. Deutsch, S. W. Jamieson, P. A. Thistlethwaite, Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N. Engl. J. Med.* **342**, 626–633 (2000).
13. W. Wong, J. D. Scott, AKAP signalling complexes: Focal points in space and time. *Nat. Rev. Mol. Cell Biol.* **5**, 959–970 (2004).
14. K. L. Dodge, S. Khouangsathiene, M. S. Kapiloff, R. Mouton, E. V. Hill, M. D. Houslay, L. K. Langeberg, J. D. Scott, mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. *EMBO J.* **20**, 1921–1930 (2001).
15. S. H. Soderling, K. L. Binns, G. A. Wayman, S. M. Davee, S. H. Ong, T. Pawson, J. D. Scott, The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. *Nat. Cell Biol.* **4**, 970–975 (2002).
16. D. Diviani, J. Soderling, J. D. Scott, AKAP-Lbc anchors protein kinase A and nucleates $G\alpha_{12}$–selective Rho-mediated stress fiber formation. *J. Biol. Chem.* **276**, 44247–44257 (2001).
17. I. D. Fraser, M. Cong, J. Kim, E. N. Rollins, Y. Daaka, R. J. Lefkowitz, J. D. Scott, Assembly of an A kinase–anchoring protein–β2-adrenergic receptor complex facilitates receptor phosphorylation and signaling. *Curr. Biol.* **10**, 409–412 (2000).
18. J. J. Michel, I. K. Townley, K. L. Dodge-Kafka, F. Zhang, M. S. Kapiloff, J. D. Scott, Spatial restriction of PDK1 activation cascades by anchoring to mAKAPα. *Mol. Cell* **20**, 661–672 (2005).
19. B. Lygren, C. R. Carlson, K. Santamaria, V. Lissandron, T. McSorley, J. Litzenberg, D. Lorenz, B. Wiesner, W. Rosenthal, M. Zaccolo, K. Taskén, E. Klussmann, AKAP complex regulates Ca^{2+} re-uptake into heart sarcoplasmic reticulum. *EMBO Rep.* **8**, 1061–1067 (2007).
20. M. F. Navedo, M. Nieves-Cintron, G. C. Amberg, C. Yuan, V. S. Votaw, W. J. Lederer, G. S. McKnight, L. F. Santana, AKAP150 is required for stuttering persistent Ca^{2+} sparklets and angiotensin II–induced hypertension. *Circ. Res.* **102**, e1–e11 (2008).
21. G. K. Carnegie, J. Soughayer, F. D. Smith, B. S. Pedroja, F. Zhang, D. Diviani, M. R. Bristow, M. T. Kunkel, A. C. Newton, L. K. Langeberg, J. D. Scott, AKAP-Lbc mobilizes a cardiac hypertrophy signaling pathway. *Mol. Cell* **32**, 169–179 (2008).
22. C. J. Schofield, P. J. Ratcliffe, Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell Biol.* **5**, 343–354 (2004).
23. M. E. Lieb, K. Menzies, M. C. Moschella, R. Ni, M. B. Taubman, Mammalian *EGLN* genes have distinct patterns of mRNA expression and regulation. *Biochem. Cell Biol.* **80**, 421–426 (2002).
24. E. Berra, E. Benizri, A. Ginouvès, V. Volmat, D. Roux, J. Pouysségur, HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1α in normoxia. *EMBO J.* **22**, 4082–4090 (2003).
25. R. J. Appelhoff, Y.-M. Tian, R. R. Raval, H. Turley, A. L. Harris, C. W. Pugh, P. J. Ratcliffe, J. M. Gleadle, Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J. Biol. Chem.* **279**, 38458–38465 (2004).
26. G. Hu, E. R. Fearon, Siah-1 N-terminal RING domain is required for proteolysis function, and C-terminal sequences regulate oligomerization and binding to target proteins. *Mol. Cell Biol.* **19**, 724–732 (1999).
27. G. Polekhina, C. M. House, N. Traficante, J. P. Mackay, F. Relaix, D. A. Sassoon, M. W. Parker, D. D. Bowtell, Siah ubiquitin ligase is structurally related to TRAF and modulates TNF-α signaling. *Nat. Struct. Biol.* **9**, 68–75 (2002).
28. K. L. Dodge-Kafka, J. Soughayer, G. C. Pare, J. J. Carlisle Michel, L. K. Langeberg, M. S. Kapiloff, J. D. Scott, The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature* **437**, 574–578 (2005).
29. B. L. Ebert, J. D. Firth, P. J. Ratcliffe, Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter–1 via distinct cis-acting sequences. *J. Biol. Chem.* **270**, 29083–29089 (1995).
30. J. A. Forsythe, B. H. Jiang, N. V. Iyer, F. Agani, S. W. Leung, R. D. Koos, G. L. Semenza, Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell Biol.* **16**, 4604–4613 (1996).
31. G. C. Pare, J. L. Easlick, J. M. Mislow, E. M. McNally, M. S. Kapiloff, Nesprin-1α contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp. Cell Res.* **303**, 388–399 (2005).
32. G. L. Semenza, F. Agani, D. Feldser, N. Iyer, L. Kotch, E. Laughner, A. Yu, Hypoxia, HIF-1, and the pathophysiology of common human diseases. *Adv. Exp. Med. Biol.* **475**, 123–130 (2000).
33. M. Colledge, E. M. Snyder, R. A. Crozier, J. A. Soderling, Y. Jin, L. K. Langeberg, H. Lu, M. F. Bear, J. D. Scott, Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron* **40**, 595–607 (2003).
34. A. Carlucci, A. Adornetto, A. Scorziello, D. Viggiano, M. Foca, O. Cuomo, L. Annunziato, M. Gottesman, A. Feliciello, Proteolysis of AKAP121 regulates mitochondrial activity during cellular hypoxia and brain ischaemia. *EMBO J.* **27**, 1073–1084 (2008).
35. M. S. Kapiloff, R. V. Schillace, A. M. Westphal, J. D. Scott, mAKAP: An A-kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes. *J. Cell Sci.* **112**, 2725–2736 (1999).

36. K. Tasken, E. M. Aandahl, Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol. Rev.* **84**, 137–167 (2004).
37. R. S. Westphal, S. J. Tavalin, J. W. Lin, N. M. Alto, I. D. Fraser, L. K. Langeberg, M. Sheng, J. D. Scott, Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* **285**, 93–96 (1999).
38. N. Hoshi, L. K. Langeberg, J. D. Scott, Distinct enzyme combinations in AKAP signalling complexes permit functional diversity. *Nat. Cell Biol.* **7**, 1066–1073 (2005).
39. A. L. Bauman, J. Soughayer, B. T. Nguyen, D. Willoughby, G. K. Carnegie, W. Wong, N. Hoshi, L. K. Langeberg, D. M. Cooper, C. W. Dessauer, J. D. Scott, Dynamic regulation of cAMP synthesis through anchored PKA-adenylyl cyclase V/VI complexes. *Mol. Cell* **23**, 925–931 (2006).
40. D. Willoughby, W. Wong, J. Schaack, J. D. Scott, D. M. Cooper, An anchored PKA and PDE4 complex regulates subplasmalemmal cAMP dynamics. *EMBO J.* **25**, 2051–2061 (2006).
41. B. J. Tunquist, N. Hoshi, E. S. Guire, F. Zhang, K. Mullendorff, L. K. Langeberg, J. Raber, J. D. Scott, Loss of AKAP150 perturbs distinct neuronal processes in mice. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 12557–12562 (2008).
42. M. Sano, T. Minamino, H. Toko, H. Miyauchi, M. Orimo, Y. Qin, H. Akazawa, K. Tateno, Y. Kayama, M. Harada, I. Shimizu, T. Asahara, H. Hamada, S. Tomita, J. D. Molkentin, Y. Zou, I. Komuro, p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature* **446**, 444–448 (2007).
43. R. L. Nicol, N. Frey, G. Pearson, M. Cobb, J. Richardson, E. N. Olson, Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy. *EMBO J.* **20**, 2757–2767 (2001).
44. X. Pi, G. Garin, L. Xie, Q. Zheng, H. Wei, J. Abe, C. Yan, B. C. Berk, BMK1/ERK5 is a novel regulator of angiogenesis by destabilizing hypoxia inducible factor 1α. *Circ. Res.* **96**, 1145–1151 (2005).
45. N. Masson, C. Willam, P. H. Maxwell, C. W. Pugh, P. J. Ratcliffe, Independent function of two destruction domains in hypoxia-inducible factor-α chains activated by prolyl hydroxylation. *EMBO J.* **20**, 5197–5206 (2001).
46. B. Hu, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, M. W. Kilimann, The paralemmin protein family: Identification of paralemmin-2, an isoform differentially spliced to AKAP2/AKAP-KL, and of palmdelphin, a more distant cytosolic relative. *Biochem. Biophys. Res. Commun.* **285**, 1369–1376 (2001).
47. G. C. Pare, A. L. Bauman, M. McHenry, J. J. Michel, K. L. Dodge-Kafka, M. S. Kapiloff, The mAKAP complex participates in the induction of cardiac myocyte hypertrophy by adrenergic receptor signaling. *J. Cell Sci.* **118**, 5637–5646 (2005).
48. J. J. Carlisle Michel, K. L. Dodge, W. Wong, N. C. Mayer, L. K. Langeberg, J. D. Scott, PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. *Biochem. J.* **381**, 587–592 (2004).
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