EPAS1 Promotes Adipose Differentiation in 3T3-L1 Cells*

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Adipose differentiation is regulated by several transcription factors, such as the CAAT/enhancer-binding protein family and peroxisome proliferator activator (PPAR) γ2. Several recent studies have shown that the basic helix-loop-helix-PAS superfamily is also involved in the regulation of adipose differentiation. In this study, we investigated the roles played by EPAS1 (endothelial PAS domain protein 1) in adipogenesis. EPAS1, also referred to as hypoxia-inducible factor 2α, is a transcription factor known to play essential roles in catecholamine homeostasis, vascular remodeling, and the maintenance of reactive oxygen species, and so forth. During adipose differentiation in 3T3-L1 cells, the level of EPAS1 mRNA began to increase 6 days after the induction, and EPAS1 was highly expressed in differentiated cells. To examine whether EPAS1 is involved in adipogenesis, we first isolated stable clones from 3T3-L1 cells in which we could induce the expression of an EPAS1 C-terminal deletion mutant (designated EPAS1-(1–485)) with the insect hormone. The induction of EPAS1-(1–485) allowed the cells to accumulate only minimum amounts of intracellular lipid droplets. Consistent with the morphological observations, a minimum amount of αP2 and PPARγ2 mRNA was induced in the EPAS1-(1–485) cells. We then examined whether or not EPAS1 was able to promote adipogenesis in NIH 3T3 cells, a relatively nonadipogenic cell line. Overexpression of EPAS1 in NIH 3T3 cells induced a significant amount of lipid accumulation compared with that of the control cells in the presence of the PPARγ ligand. The results were also confirmed by measuring the expression of adipocyte-related genes. Adenovirus-mediated EPAS1-(1–485) expression resulted in the reduction of basal and insulin-dependent glucose transport in 3T3-L1 adipocytes. The mechanism involved the transcriptional regulation of GLUT1, GLUT4, and IRS3 expression by EPAS1. Taken together, these results suggest that EPAS1 plays several supporting roles in maintaining specific aspects of adipogenesis and adipocyte function including regulation of glucose uptake followed by lipid synthesis.

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# The abbreviations used are: C/EBP, CAAT/enhancer-binding protein; PPAR, peroxisome proliferator activator; bHLH, basic helix-loop-helix; HIF, hypoxia-inducible factor; PonA, ponasterone A.

Adipose differentiation is a complex process by which fibroblast-like undifferentiated cells are converted into cells that accumulate lipid droplets. Recent studies using DNA arrays revealed that expression of several hundred genes is altered in this process (1). This complex differentiation process is driven by the coordinated expression of various transcription factors, including the CAAT/enhancer-binding protein (C/EBP) family and peroxisome proliferator activator (PPAR) γ2 (2–4). In preadipocytes, the Wnt signaling pathway is part of the machinery, which maintains the cells in undifferentiated states (5). Adipogenesis begins on the treatment of preadipocytes with fetal calf serum, insulin, dexamethasone, and an inducer of intracellular cyclic AMP such as isobutylmethylxanthine. A rapid and transient increase in transcription and expression of C/EBPβ and C/EBPδ is observed in the early stages of differentiation. These factors have been shown to promote adipogenesis, presumably through induction of C/EBPa and PPARγ2 (2–4). The enforced expression of PPARγ2 or C/EBPa has been shown to stimulate adipogenesis in NIH 3T3 fibroblasts (6–8), suggesting the essential roles played by these factors in regulating adipogenesis in vitro. Cells lacking PPARγ2 had a greatly reduced level of C/EBPa (9–11). Similarly, cells lacking C/EBPa have reduced adipogenic potential and expression of PPARγ2 (12). Importantly, adding PPARγ2 back to C/EBPa-null cells has been shown to restore their ability to accumulate lipids and activate markers of adipose differentiation (12). Recent studies have demonstrated that ectopic expression of C/EBPa failed to rescue the ability to differentiate in PPARγ2-null mouse embry fibroblasts (13). Therefore, rather than being an equal co-director of the adipogenesis program, PPARγ2 plays a leading role in the adipogenic hierarchy. On the other hand, C/EBPa is critical in the establishment of insulin sensitivity (12). This effect of C/EBPa is mediated in part by the direct transcriptional induction of both the insulin receptor and IRS1 (12). Consequently, C/EBPa is influential in maintaining the expression of PPARγ2 and in promoting full insulin sensitivity.

Several recent studies have shown that the basic helix-loop-helix (bHLH)-PAS superfamily is also involved in regulation of adipose differentiation (14–17). The bHLH-PAS proteins are characterized by the PAS domain, which is composed of two imperfect 50-amino acid repeats and a bHLH domain. The term “PAS” is derived from the first three members of the family: the period gene, the aryl hydrocarbon receptor nuclear translocator (ARNT), and the single-minded (SIM) gene. Using the PAS domain and the bHLH domain, proteins of this family form heterodimers that bind to a target gene through the basic region and govern the functions of that gene. The PAS domain...
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In the context of adipogenesis, EPAS1, also referred to as HIF-2, plays a role in the regulation of adipose differentiation. Consequently, several bHLH-PAS proteins are thought to participate in this process.

Conversely, hypoxia inhibits adipogenesis in a hypoxia-inducible factor (HIF)-1α-dependent manner. The putative mechanism for this process is that HIF-1α-regulated gene Dec 1/Sta I3 represses PPARγ2 promoter activity.

Studies using EPAS1-null mice revealed that EPAS1 is essential for catecholamine homeostasis and vascular remodeling. More recently, Scortegagna et al. (27) revealed that EPAS1 plays a role in the maintenance of reactive oxygen species. In addition to these roles, a contribution of EPAS1 to the regulation of adipogenesis has been suggested for the following reasons.

First, several growth factors, including insulin and insulin-like growth factor, induce and activate EPAS1 expression in a phosphorylation-dependent manner. Also, HIF-1α, which is closely related to HIF-2α, HLF, or MOP2, is a transcription factor predominantly expressed in endothelial cells and the organ of Zuckerland (22–25).

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the expression level of EPAS1 in mice adipose tissue. In white adipose tissue isolated from C57BL/6J mice, EPAS1 was predominantly expressed in fractions containing adipocytes, as compared with stromal-vascular fractions (Fig. 1A). Also, white adipose tissue in mice fed a high-fat diet expressed a much higher level of EPAS1 mRNA compared with that in control mice (Fig. 1B). These results suggest that the expression of EPAS1 is increased during adipose differentiation in vivo. EPAS1 was originally identified as the transcriptional factor abundantly expressed in endothelial cells (22–24). Thus we compared the expression level of EPAS1 in adipocytes with that in endothelial cells. The level of EPAS1 mRNA expression
in adipocytes is as high as that in endothelial cells (Fig. 1C). Then, in the next set of experiments, preadipose 3T3-L1 cells were differentiated to adipocytes by the addition of dexamethasone, isobutylmethylxanthine, insulin, and fetal bovine serum, and the expression of EPAS1 during adipogenesis was analyzed by Western blotting (Fig. 1D). The adipose differentiation status was confirmed by staining with Oil Red O and by measurement of glyceraldehyde-3-phosphate dehydrogenase activity 8 days after induction (data not shown). The level of EPAS1 protein began to increase 6 days after induction and was markedly expressed in the differentiated cells (8 days after induction). Similar to the protein expression levels, EPAS1 mRNA levels increased during adipose differentiation (Fig. 1E). Conversely, expression of HIF-1α was detected only at the early stage and declined during adipose differentiation (Fig. 1E). To characterize the expression of EPAS1 during adipogenesis, the effects of adipocyte-related transcription factors such as C/EBPs and PPARγ on EPAS1 promoter activity were examined (Fig. 1F). Luciferase activity driven by the EPAS1 promoter was increased by C/EBPβ or C/EBPδ, whereas C/EBPα and PPARγ had little or no effect on this activity (Fig. 1F).

Expression of the Dominant Negative Form of EPAS1 Inhibits Adipose Differentiation in 3T3-L1 Cells—To examine whether EPAS1 exerts an influence on adipogenesis, we first constructed an EPAS1 C-terminal deletion mutant (designated EPAS1-(1–485)) that lacked a transactivation domain. Although EPAS1-(1–485) forms a heterodimer with ARNT and binds to the hypoxia-responsive element sequence (32). However, this mutated EPAS1 lacks the ability to transactivate HRE-driven transcription (Fig. 2A; Ref. 32). Co-expression of EPAS1-(1–485) suppressed the induction of HRE-driven transcription by full-length EPAS1 in a dose-dependent manner (Fig. 2A), indicating that EPAS1-(1–485) functioned as a dominant negative mutant. Although EPAS1-(1–485) also inhibits HIF-1α-dependent transcriptional activity in vitro (data not shown), the expression pattern of EPAS1 is distinct from that of HIF-1α during adipogenesis (Fig. 1E). Furthermore, HIF-1α protein is unstable due to oxygen-dependent ubiquitination under normoxia conditions, whereas EPAS1 protein is expressed at constant levels regardless of oxygenation (33, 34). Therefore, the effects of EPAS1-(1–485) observed in this study are most likely...
on EPAS1 activity, but not HIF-1α activity. We then isolated stable clones from 3T3-L1 cells in which we could induce the expression of EPAS1-(1–485) with insect hormone (Pon.A) (Fig. 2B). This system allows us to directly examine the roles of EPAS1 without relying on pharmacological agents or hypoxia conditions that might regulate other signaling pathways and transcriptional activities. Treatment of the control 3T3-L1 cells with Pon.A had no effect on the differentiation states, as judged by Oil Red O staining (Fig. 2C). Conversely, the induction of EPAS1-(1–485) allowed cells to accumulate only minimum amounts of intracellular lipid droplets (Fig. 2, C and D). These results were confirmed by measuring the expression of adipocyte-related genes, such as PPARγ2 and the C/EBP family. As expected, the expression of these genes was strongly induced in the control cells during differentiation (Fig. 2D). In contrast, consistent with the morphological observations, minimum amounts of PPARγ2 and C/EBP family were induced in the EPAS1-(1–485)-expressing cells. Consequently, these results indicated that EPAS1 plays a role for execution of the adipose differentiation program in 3T3-L1 cells.

The Combination of the PPARγ Activator and a Conventional Differentiation Mixture Restores the Ability of EPAS1-(1–485)-expressing Cells to Differentiate—Activation of PPARγ with ligands such as thiazolidinediones can promote adipose differentiation. Therefore, we chose to examine whether or not the activation of PPARγ would be able to restore the differentiation potential of EPAS1-(1–485)-expressing cells. Consistent with the results shown in Fig. 2, the induction of EPAS1-(1–485) with PonA resulted in a lower differentiation morphology (Fig. 3, middle row). However, treatment with troglitazone allowed the EPAS1(1–485)-expressing cells to accumulate lipid droplets to the same extent as that of the control cells in the presence of PonA (Fig. 3, bottom row). Similarly, co-treatment with the conventional differentiation mixture containing ciglitazone or indomethacin restored the ability of the cells to differentiate (data not shown).

Ectopic Expression of EPAS1 in NIH 3T3 Cells Stimulates Adipose Differentiation—The results described above demonstrated that EPAS1 plays a role in adipogenesis in 3T3-L1 cells. Then, we wished to examine whether EPAS1 has the ability to promote adipogenesis in NIH 3T3 cells, a relatively nondiabeticogenic cell line. NIH 3T3 cells were stably transfected with either a control vector or a vector expressing high levels of full-length EPAS1 mRNA. After the selection and expansion of...
stable clones, the level of the EPAS1 protein was examined by Western blot analysis. As shown in Fig. 4A, the cells transfected with the vector expressing EPAS1 mRNA produced more EPAS1 protein than did the control vector-transfected cells. To evaluate the differentiation potency of the clones, the cells were cultured to confluence and then treated with a standard differentiation medium containing PPARγ2 ligand (troglitazone or 5,8,11,14-eicosatetraynoic acid). The extent of differentiation was estimated by adipose staining with Oil Red O. Neither the EPAS1-expressing cell clones nor the control cells showed signs of lipid accumulation when cultured in the absence of differentiation-inducing agents (data not shown). Treatment with adipose differentiation medium containing PPARγ2 ligands enabled the wild-type NIH 3T3 cells and the cells expressing vector mRNA to exhibit only a minimum degree of lipid accumulation (Fig. 4B and C). However, the cells overexpressing EPAS1 showed more lipid accumulation than was observed in the control cells 10 days after induction of differentiation (Fig. 4B and C). The expression of adipocyte-related genes, such as PPARγ2 and aP2, was greatly induced in the EPAS1-expressing cells, whereas these genes were poorly expressed in the control cells (Fig. 4D).

**EPAS1 Regulates Glucose Uptake in Adipocytes**—In addition to PPARγ2, insulin plays pivotal roles in adipose differentiation. Previous studies have shown that insulin-like growth factor activates EPAS1 in osteoblast-like cells (28). Thus, to understand the molecular basis by which EPAS1 promotes adipose differentiation, we next examined the effects of EPAS1-(1–485) on the expression of genes for insulin signaling and glucose transport. As described in Fig. 2, the induction of EPAS1-(1–485) in preadipocytes resulted in failure of differentiation, and therefore it is not clear whether alterations of gene expression in EPAS1-(1–485)-expressing cells during adipose differentiation reflect direct effects of EPAS1-(1–485) or are an indirect result of the lower level of differentiation of EPAS1-(1–485) cells. Thus, in these experiments, 3T3-L1 cells were induced to differentiate, and mature adipocytes were infected with adenovirus expressing either LacZ or EPAS1-(1–485). As shown in Fig. 5 (−), infection with adenovirus carrying LacZ had no effect on expression of the genes examined. Overexpression of EPAS1-(1–485) in 3T3-L1 adipocytes dramatically suppressed the expression of GLUT1, GLUT4, and IRS3, whereas the expression of other genes such as PPARγ2, aP2, insulin receptor (IR), IRS1, and GLUT8 was not significantly altered (Fig. 5). We then examined the ability of EPAS1 to directly regulate the transcriptional activity of these genes. The luciferase reporter gene driven by the promoter/enhancer of GLUT1, GLUT4, IRS3, and PPARγ2 was constructed and

**Fig. 6.** EPAS1 activates the promoter activity of GLUT1 (A), GLUT4 (B), and IRS3 (C), but not that of PPARγ2 (D). Full-length EPAS1 and ARNT expression plasmids were transfected into HEK 293 cells with the reporter plasmid (0.15 μg) carrying the promoter region of the indicated genes. In D, the PPARγ2 promoter-driven luciferase plasmid (0.15 μg) was transfected with a pair of full-length EPAS1 (0.15 μg) and ARNT expression plasmids (0.15 μg) or C/EBPα expression plasmid (0.15 μg) as a positive control. For all constructs, pRL-SV40 vector (Promega) was co-transfected to correct for differences in transfection efficiency, and the normalized luciferase activity was represented as arbitrary units. The averages of three independent experiments are shown.
transfected into 293 cells with different amount of EPAS1 expression vector. As shown in Fig. 6A–C, EPAS1 enhanced the promoter/enhancer activity of GLUT1, GLUT4, and IRS3 genes in a dose-dependent manner. Also, consistent with the mRNA expression results (Fig. 5), EPAS1/ARNT was found to have no effect on PPARγ promoter activity (Fig. 6D). To evaluate whether these changes in gene expression had an effect on the biological activity of adipocytes, glucose transport activity in EPAS1-(1–485)-expressing adipocytes was determined. In the absence of insulin, the amount of glucose transported into EPAS1-(1–485)-expressing cells was <50% of that in the control cells (Fig. 7A). Although the addition of insulin increased the amount of glucose transported into the cells, the glucose transport activity of the EPAS1-(1–485)-expressing cells was still significantly lower than that of the control cells (Fig. 7A). In contrast to glucose transport activity, the degree of Akt phosphorylation was the same in EPAS1-(1–485)-expressing cells and control cells (Fig. 7B).

**DISCUSSION**

EPAS1 was originally identified as a transcription factor response to hypoxia conditions (21–23). Although EPAS1 and HIF-1α share high amino acid homology, they may have distinct functions because of differences in tissue distribution and in the developmental expression profiles of these proteins (21–23, 35, 36). A recent study has revealed that the target genes of EPAS1 are, at least in part, distinct from those of HIF-1α (37). More interestingly, oxygen-dependent protein degradation is restricted to HIF-1α (33). In many cell lines such as mouse embryo fibroblasts, EPAS1 escapes oxygen-dependent protein degradation and is no longer a hypoxia-inducible factor (34). Together with the results from knockout mice studies, these results strongly suggest that EPAS1 plays a critical role in embryonic development and homeostasis under normoxia conditions (25–27). In this study, we demonstrated that EPAS1 is highly induced during adipose differentiation in vivo and in vitro (Fig. 1). Also, the C/EBP family can activate EPAS1 promoter activity in vitro (Fig. 1D). Although little has been known about the role of this factor in adipose differentiation, the present observations suggest that EPAS1 may play a role in adipogenesis. The expression of a dominant negative form of EPAS1 suppressed the morphological differentiation of 3T3-L1 cells, as well as induction of adipocyte-related genes (Fig. 2). Conversely, overexpression of full-length EPAS1 in nondiagenic NIH 3T3 cells facilitated lipid droplet accumulation in the cells (Fig. 4), although the presence of the PPARγ ligand is required for adipose differentiation of EPAS1-expressing NIH 3T3 cells (data not shown). The morphological differentiation in EPAS1-expressing NIH 3T3 cells was accompanied by the induction of adipogenic markers such as PPARγ2 and aP2 (Fig. 4). Finally, DEXA scan analysis revealed that adipose tissues in EPAS1 knockout mice are smaller than those in control mice. Consequently, EPAS1 appears to play a role in promotion of adipose differentiation.

In this study, the results obtained using the luciferase reporter assay indicate that the EPAS1 promoter is differentially regulated by different C/EBPs (Fig. 1F). A number of other studies show differential action of C/EBPα and C/EBPβ in activating various promoters including PPARγ2 (38–42). Although precise analysis on the EPAS1 promoter has not yet been performed, computer analysis revealed that the putative binding sites of C/EBPs on the EPAS1 promoter region are not identical to those found in PPARγ2 (data not shown). These results, taken together, support idea that the action of C/EBPs involves context-specific effects and depends on promoter composition as reported previously (43).

Adipose differentiation is a complex process accompanied by the alteration of expression of several hundred genes (1). The coordination of this complex process is driven mainly by PPARγ2 (4). Although the induction of a dominant negative form of EPAS1 in 3T3-L1 preadipocytes reduced the expression level of PPARγ2 (Fig. 2D), this may be an indirect result of the lower level of differentiation of EPAS1-(1–485) cells because of the following reasons. First, in mature adipocytes, dominant negative EPAS1 had no effect on PPARγ2 expression (Fig. 5). Second, the early expression level of PPARγ2 in EPAS1-expressing NIH 3T3 cells was not substantially different from that in the control cells (Fig. 4D). Moreover, EPAS1 had no effect on PPARγ2 promoter activity (Fig. 6D). Therefore, it is unlikely that PPARγ2 is a direct target of EPAS1.

We demonstrated in this study that EPAS1 regulates both basal and insulin-dependent glucose transport into cells (Fig. 7A). EPAS1-(1–485) had no effects on phosphorylation of Akt (Fig. 7B), suggesting that EPAS1 is not likely to be involved in regulation of the Akt signaling pathway. Therefore, the mechanism by which EPAS1 regulates glucose uptake may be the direct transcriptional regulation of several factors including GLUT1 and GLUT4 (Figs. 5 and 6), although we cannot exclude the possibility that EPAS1-(1–485) suppressed the translocation activity of the glucose transporter. Several lines of evidence have suggested that these factors play pivotal roles in the promotion of adipose differentiation. A previous study using transgenic mice revealed that GLUT4 regulates the num-
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number of fat cells as well as insulin sensitivity (44). GLUT4-null mice exhibit decreased longevity associated with cardiac hypertrophy and severely reduced adipose tissue deposits (45). GLUT1 expression and GLUT4 translocation can be regulated by PPAR (46), suggesting that restoration of the ability of EPAS1 (1–485) cells to differentiate (Fig. 3) by troglitazone is likely due to the recovery of these factors. If this is indeed the case, the present results support the notion that the reduced expression of GLUT1 and GLUT4 is partly responsible for the reduced differentiation potency of EPAS1 (1–485) cells. The promotion of glucose flux and subsequent glycolysis by these factors are known to contribute to an increase in lipid synthesis in adipocytes (47, 48). In addition to GLUT1 and GLUT4, IRS3 could be the target gene of EPAS1 (Figs. 5 and 6). White adipose tissue of Irs1/–/Irs3/– double knockout mice was reported an alteration of serum acyl-carnitine profiles in EPAS1-null mice. The markedly high C16:C2 ratio and the spectrum of intermediate acyl-fatty acids species in EPAS1-null mice suggest that EPAS1 participates in the regulation of lipid metabolism (27). Consequently, we are led to conclude that EPAS1 plays several supporting roles in maintaining specific aspects of adipogenesis and adipocyte function, such as regulation of glucose uptake followed by lipid synthesis, despite not being directly responsible for the induction of adipogenesis per se.

REFERENCES
1. Souchas, A., Socci, N. D., Saatkamp, B. D., Novelli, S., and Friedman, J. M. (2001) J. Biol. Chem. 276, 34167–34174
2. Cornelius, P., MacDougall, O. A., and Lane, M. D. (1994) Annu. Rev. Biochem. 64, 345–373
3. Tontonz, P., Hu, E., and Spiegelman, B. M. (1994) Curr. Opin. Genet. Dev. 5, 571–576
4. Ross, S. E., Hemati, N., Longo, K. A., Bennett, C. N., Lucas, P. C., Erickson, R. L., and MacDougall, O. A. (2000) Science 289, 950–953
5. Lin, P.-T., and Lane, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8757–8761
6. Freytag, S. O., Pielli, D. L., and Gilbert, J. D. (1994) Genes Dev. 8, 1654–1663
7. Tontonz, P., Hu, E., and Spiegelman, B. M. (1994) Cell 79, 1147–1156
8. Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lunano, P., Chien, K. R., Koder, A., and Evans, R. M. (1999) Mol. Cell 4, 585–595
9. Kukita, T., Terauchi, Y., Yamauchi, T., Komeda, K., Miki, H., Tamemoto, H., and Spiegelman, B. M. (1999) Mol. Cell 4, 611–617
10. Wu, Z., Rosen, E. D., Brun, R., Hauser, S., Adelman, G., Troy, A. E., Mclean, C., Darlington, G. J., and Spiegelman, B. M. (1999) Mol. Cell 3, 151–158
11. Rosen, E. D., Sarraf, P., Troy, A. E., Bradwin, G., Moore, K., Milstone, D. S., Spiegelman, B. M., and Mortensen, R. M. (1999) Mol. Cell 4, 611–617
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