Phosphorylation by Mitogen-activated Protein Kinase Mediates the Hypoxia-induced Turnover of the TAL1/SCL Transcription Factor in Endothelial Cells*

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The basic helix-loop-helix transcription factor TAL1 (or SCL), originally identified from its involvement by a chromosomal rearrangement in T-cell acute lymphoblastic leukemia, is required for hematopoietic development. TAL1 also has a critical role in embryonic vascular remodeling and is expressed in endothelial cells postnatally, although little is known about its function or regulation in this cell type. We report here that the important proangiogenic stimulus hypoxia stimulates phosphorylation, ubiquitination, and proteasomal breakdown of TAL1 in endothelial cells. Tryptic phosphopeptide mapping and chemical inhibitor studies showed that hypoxia induced the mitogen-activated protein kinase-mediated phosphorylation of a single serine residue, Ser122, in the protein, and site-directed mutagenesis demonstrated that Ser122 phosphorylation was necessary for hypoxic acceleration of TAL1 turnover in an immortalized murine endothelial cell line. Finally, whereas TAL1 expression was detected in endothelial cells from both large and small vessels, hypoxia-induced TAL1 turnover was observed only in microvascular endothelial cells. Besides their implications for TAL1 function in angiogenic processes, these results demonstrate that a protein kinase(s) important for mitogenic signaling is also utilized in hypoxic endothelial cells to target a transcription factor for destruction.

TAL1 binds DNA with any of the more widely expressed basic helix-loop-helix proteins known as E proteins, including the E12, E47, and E2-5 splice isoforms of the E2A gene, the related E2-2 protein, and the HEB/HTF4 gene products (10, 11). These TAL1-E protein heterodimers recognize a nucleotide motif, CANNTG, termed the E-box to activate or repress transcription (12–15). A variety of adaptor proteins or coregulators, including the LIM domain oncoproteins LMO1 and LMO2 (16, 17), histone acetyltransferases p300/CBP (14) and P/CAF (18), and nuclear corepressors mSin3A and mSin3B (15), also interact with TAL1 and modulate its transcriptional properties. With rare exceptions (19), however, TAL1’s target genes remain unidentified.

TAL1 is a critical regulator of hematopoietic differentiation. Tal1 gene inactivation by homologous recombination resulted in midgestational lethality with the complete absence of yolk sac erythropoiesis (20, 21). From analysis of chimeric animals derived from mixtures of Tal1+/− and Tal1−/− embryonic stem cells, the gene also appears to be required for generation of lympho-hematopoietic cells of the adult (22, 23). In addition, TAL1 promotes the terminal differentiation of specific hematopoietic lineages (24–26).

Although less well characterized, TAL1 also has a role in blood vessel formation or maturation. Tal1 protein has been observed in endothelial progenitor cells, or angioblasts, and in endothelial and hematopoietic cells of the blood islands of the embryonic yolk sac (27, 28). Furthermore, enforced expression of TAL1 in zebrafish embryos resulted in overproduction of both hematopoietic and endothelial precursors (29), and studies of Tal1−/− embryos in which hematopoiesis was partially rescued by a Gata-1 promoter-driven Tal1 transgene revealed the gene is also required for vascular remodeling (30). Finally, Tal1 expression characterizes vascular and lymphatic endothelial cells of the adult (31). However, its specific actions in this cell type and the extent to which its expression can be modulated by angiogenic molecules are not known.

We used an immortalized murine endothelial cell line to investigate the effects of an important proangiogenic stimulus on Tal1 protein dynamics. We found that hypoxia greatly accelerated Tal1 turnover in these cells through mitogen-activated protein kinase (MAPK)-mediated phosphorylation,
ubiquitination, and proteasomal degradation. In addition to their implications for TAL1’s actions in angiogenesis, these studies show that a protein kinase(s) important for mitogenic signaling can also be employed in targeting a transcription factor for destruction.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The MS1 cell line was generated by transduction of pancreatic microvascular endothelial cells with a temperature-sensitive simian virus 40 large T antigen (32). Human umbilical vein endothelial cells (HUVECs) and human dermal microvascular endothelial cells (HMVECs) were purchased from Clonetech (San Diego, CA), and COS-7 and HeLa cells were obtained from the American Type Culture Collection (Manassas, VA). The immortalized human bone marrow microvascular endothelial cell line BMEC-1 (33) was provided by Francisco Redman, Richmond, CA). COS-7, HeLa, and murine erythroblastemia cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) with 5% heat-inactivated fetal bovine serum (FBS) in a humidified 10% CO2 atmosphere at 37 °C. HUVECs were cultured in endothelial growth medium (EGM; Clonetics), and HMVECs were grown in microvascular endothelial growth medium (EGM-MV; Clonetics). COS-7, HeLa, and murine erythroblastemia cells were cultured in Dulbecco’s modified Eagle’s medium with 10% heat-inactivated FBS, and EGM-Eagle Medium with 15% FBS, 4% methylcellulose endothelial cell growth supplement (Sigma), 1 mM t-glutamine, 10 mM HEPES, 16 units/ml heparin, and 25 mM sodium bicarbonate. To induce hypoxia, tissue culture dishes were transferred to a Billups-Rothenberg chamber, which was flushed with a defined gas mixture, sealed, and returned to a 37 °C incubator (34). These mixtures contained 10% CO2 and either 0%, 0.5%, or 1.0% O2.

**Plasmids**—An expression plasmid (pT123) for influenza virus hemagglutinin (HA) epitope-tagged ubiquitin (35) was provided by Dirk Bohmann (European Molecular Biology Laboratory, Heidelberg, Germany). Construction of pcDNA-Tal1 and pcDNA1-Tal111222 plasmids has been described previously (36). To express Myc epitope-tagged Tal1 and Tal111222 proteins, the appropriate cDNA was subcloned into vector pcDNA1.1 (+) (Invitrogen), and a nucleotide sequence encoding a human c-Myc epitope was inserted by site-directed mutagenesis immediately before the stop codon (GeneEditor In Vitro Site-Directed Mutagenesis System, Promega, Madison, WI).

**Protease Inhibitors**—Protease inhibitors MG132 (N-carboxybenzoyl-L-arginyl-L-glutamyl-L-lysine; Sigma), LLM (N-acetyl-L-arginyl-L-glutamyl-L-lysine; Sigma), LmN-L (N-acetyl-L-arginyl-L-glutamyl-L-lysine; Sigma), and E64 (trans-epoxysuccinyl-L-lysylamide-O-guanidino)buthane; Sigma) were dissolved in MeSO before addition to culture.

**Antibodies**—Affinity-purified rabbit polyclonal antibody to mouse Tal1 has been described previously (28). Rabbit polyclonal antibody to human TAL1 was provided by Richard Baer (Columbia University, New York, NY). Purified mouse monoclonal antibody to the HA epitope (16B12) was purchased from Antibodies to CO (now Covance Research Products, Richmond, CA), mouse monoclonal antibody to HIP-1 (NE 100–123) was purchased from Novus Biologicals (Littleton, CO), and mouse monoclonal antibody 9E10 to the human c-Myc epitope was purchased from Sigma.

**Western Blot Analysis**—Nuclear extracts were prepared by the method of Dignam et al. (37) and denatured by heating at 95 °C for 3 min in Laemmli buffer (38). Samples were subjected to SDS-PAGE in 10% polyacrylamide gels. After electrophoresis, proteins were electrotransferred to a polyvinylidene difluoride membrane (Bio-Rad), which was incubated in blocking buffer (5% milk, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) at 4 °C for 4 h and then incubated with the indicated antibodies overnight. Binding of the primary antibody was detected by an enhanced chemiluminescence method (ECL Plus; Amersham Biosciences) using horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma) or sheep anti-mouse IgG (Amersham Biosciences).

**Protein Stability Analysis**—For pulse-chase analysis, MS1 cells were grown in 10-cm-diameter dishes, washed once with methionine- and cysteine-free Dulbecco’s modified Eagle’s medium (Invitrogen), and incubated at 37 °C for 4 h in this medium plus 5% dialyzed FBS. Cells were then labeled with 200 μCi/ml [35S]methionine and cysteine (Trans-35S-Lab; ICN, Irvine, CA) at 37 °C for 1 h, cells were washed with phosphate-buffered saline and chased with Dulbecco’s modified Eagle’s medium with 5% FBS under hypoxic or normoxic conditions at 37 °C. Cells were then lysed in 1 ml/dish radioluminescence precipitation assay (RIPA) buffer (9.1 mM Na2HPO4, 1.7 mM Na2HPO4, pH 7.4, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1.0% Nonidet P-40, and 1 mM dithiothreitol) containing 50 μM phenylmethylsulfonyl fluoride, 10 μg/ml pestatin, 10 μg/ml antipain, 10 μg/ml leupeptin, and 50 μg/ml aprotinin. The resulting lysates were clarified by centrifugation, and radiolabeled Tal1 was immunoprecipitated with a specific antibody. Immune precipitates were fractionated by SDS-PAGE, and band intensities were quantitated from phosphorimaging of dried gels (Molecular Dynamics, Sunnyvale, CA).

Protein half-life was also determined by Western blot analysis after treatment of cells with the protein synthesis inhibitor cycloheximide. Following a medium change, cycloheximide was added at a concentration of 100 μM, and the cells were incubated under hypoxic or normoxic conditions at 37 °C. Nuclear extracts were subjected to Western blot analysis as described above. Several treatments were used to modulate intracellular calcium levels. Calcium was removed from the medium by the Ca2+-free solution described above.

**Cell Transfections**—COS-7 and HeLa cell cultures of ~80% confluence were transfected with 10 μg of plasmid DNA by calcium phosphate coprecipitation (39), whereas subconfluent MS1 cells were transfected with 8 μg of liposome-complexed DNA (LipofectAMINE; Invitrogen). Using a β-galactosidase expression vector under identical conditions, a transfection efficiency of 60% was achieved for COS-7 cells, 80% for HeLa cells, and 10% for MS1 cells.

**In Vivo Ubiquitination Assay**—HeLa cells cultured in 10-cm-diameter dishes were transiently transfected with 4 μg of the HA-tagged ubiquitin expression vector, 4 μg of Tal1 expression vector, or both. MS1 cells were transfected with 8 μg of the HA-ubiquitin expression vector and split 24 h after transfection. After an additional 48 h, cells were treated with 10 μg MG132 for 4 h and lysed with ice-cold RIPA buffer and 10 μM phenylmethylsulfonyl fluoride. Cell lysates were clarified by centrifugation and incubated with Tal1 antibody at 4 °C overnight. The resulting immune complexes were precipitated with protein A-Sepharose (Pierce) at 4 °C for 2 h. These complexes were washed four times with ice-cold RIPA buffer, heated in Laemmli sample buffer to 95 °C for 3 min, and subjected to Western blot analysis as described above using a mouse monoclonal antibody to the HA epitope. Horseradish peroxidase-conjugated anti-mouse IgG was used to detect binding of the HA antibody.

**Metabolic Labeling of Cells and Radioimmunoprecipitation Analysis**—MS1 cells were metabolically labeled as described by Tang et al. (36). Briefly, cells were incubated in phosphate-free minimum essential medium with 5% dialyzed FBS at 37 °C for 1 h and radiolabeled with 0.5 μCi/ml [35S]orthophosphate (ICN) for 1.5 h in the presence of 10 μg/ml MG132. After treatment with 50 μg/ml PD98059 or vehicle for 30 min, cells were subjected to severe hypoxia (0% O2) or continued normoxia for 2 h. Nuclei were extracted with ice-cold RIPA buffer containing 1 mM sodium vanadate, 20 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml antipain, and 10 μg/ml pestatin A. Radiolabeled Tal1 was then immunoprecipitated from the resulting extracts with Tal1 antibody at 4 °C overnight. Immune precipitates were collected by site-directed mutagenesis immediately before the stop codon (GeneEditor In Vitro Site-Directed Mutagenesis System, Promega, Madison, WI).

**Two-dimensional Tryptic Phosphopeptide Analysis**—After immunoprecipitation and fractionation by SDS-PAGE, radiolabeled Tal1 protein was transferred to a polyvinylidene difluoride membrane, eluted, and digested exhaustively with trypsin. Proteinolytic fragments were then separated by two-dimensional phosphopeptide analysis by the method of Boyle et al. (40). Briefly, tryptic digests were applied to a cellulose thin layer electrophoresis plate (EM Science, Gibbstown, NJ) and fractionated by electrophoresis for 20 min in pH 1.9 buffer at 1000 V and by ascending thin layer chromatography in the second dimension in a solvent containing 39% butanol, 30% glyride, and 6% glacial acetic acid. Phosphorimaging was used to visualize radioactive digestion products and assess phosphorylation of specific fragments.

**MAPK Assay**—MS1 cells were transferred to serum-free medium for 12 h and lysed as described above. MAPK protein was immunoprecipitated from nuclear extracts by the method of Chen and Blenis (41) using an antibody reactive with both ERK1 and ERK2 (Upstate Biotechnology, Lake Placid, NY), and protein kinase activity was assayed as described by Robbins et al. (42). Briefly, immunoprecipitated protein was incubated with 0.2 mg/ml myelin basic protein and 100 μM [γ-32P]ATP (2 μCi/μmol) in 75 mM Tris-HCl, pH 8.0, 10 mM MgCl2 at 30 °C for 30 min. Reactions were terminated by the addition of electrophoresis sample buffer and fractionated by SDS-PAGE in a 15% gel. Myelin basic protein phosphorylation was detected by autoradiography and quantitated from phosphorimaging of the dried gel.
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RESULTS

Hypoxia Reduces Tal1 Protein Abundance in Microvascular Endothelial Cells—Hypoxia’s effect on TAL1 expression was investigated using an immortalized murine pancreatic endothelial cell line, MS1 (32). Western blot analysis revealed that Tal1 protein abundance declined significantly in MS1 cells exposed to 1.0% O2 (A), 0.5% O2 (B), or 0% O2 (C) for the times indicated, and Tal1 protein was detected by Western blot analysis as described under “Experimental Procedures.” In one experiment, cells were returned to a normal ambient oxygen concentration (C). As a control, HIF-1α protein expression was quantitated by Western blot analysis in MS1 cells that were made hypoxic (E).

Hypoxia Accelerates Tal1 Protein Turnover in Microvascular Endothelial Cells—Pulse-chase analysis showed that the decline in Tal1 protein in hypoxic MS1 cells was the result of its increased turnover (Fig. 2). Whereas Tal1 had a half-life (t1/2) of 8 h under ambient oxygen tensions, a biphasic decay curve with a t1/2 of 2 h was observed after cells were exposed to severe hypoxia. Similar results were obtained from Western blot analysis of cycloheximide-treated cells (data not shown). Moreover, Tal1 levels recovered in hypoxic cells with restoration of a normal ambient oxygen concentration (Fig. 1C). The decline in Tal1 expression was also associated with accumulation of the HIF-1α transcription factor (Fig. 1E), excluding a general increase in protein turnover.

To identify the pathway responsible for Tal1 destruction, cells were treated with different protease inhibitors before being made hypoxic, and Tal1 protein levels were then quantitated by Western blot analysis. The potent proteasomal inhibitor MG132 was tested first in Tal1-transfected HeLa cells, and, whereas hypoxia was without effect on its expression in these cells (Fig. 3A), MG132 did increase Tal1 steady-state levels. In contrast, hypoxia effected a decline in Tal1 expression in MS1 cells that was prevented by MG132 (Figs. 3B and 4) and attenuated by the less potent proteasomal inhibitor ALLN (Fig. 3B) but unaltered by the lysosomal protease inhibitor E64 and the calpain inhibitor LLM (Fig. 3B). These results thus impli-
Phosphorylation in MS1 cells made hypoxic. As shown in Fig. 5A, phosphorylation of Tal1 was virtually eliminated in cells pretreated with PD98059, providing strong evidence for the involvement of a MAPK in this process. PD98059 can inhibit MAPK kinase 5 (56, 57) in addition to MEK, so its effect on Tal1 phosphorylation could have resulted from inhibition of ERK1, ERK2, or ERK5 (56, 57).

Phosphorylation of TAL1 has been shown previously to be restricted to serine residues (58–60). Furthermore, a specific serine in the putative transactivation domain of the protein, Ser122, is preferentially phosphorylated by ERK1 in vitro (53) and is the target in cells of epidermal growth factor (EGF)- and erythropoietin-stimulated MAPK phosphorylation (36, 53). To determine which residues were phosphorylated in response to hypoxia, two-dimensional tryptic phosphopeptide analysis was carried out on radiolabeled Tal1 immunoprecipitated from hypoxic MS1 cells that had been pretreated with PD98059. As a positive control for Ser122 phosphorylation, immune precipitates were also prepared from Tal1-transfected COS-7 cells treated with EGF. Although more than one radiolabeled tryptic phosphopeptide was obtained, presumably the result of incomplete enzyme digestion, an identical fragment pattern was apparent for hypoxic MS1 cells and EGF-stimulated COS-7 cells (Fig. 5B). Because mutagenesis has shown this serine to be the only site in TAL1 of EGF-induced phosphorylation (36, 53), these results demonstrate that Ser122 is also the target of hypoxia-induced phosphorylation. Finally, nuclear MAPK activity was found to increase within 30 min after transferring MS1 cells to a hypoxic environment (data not shown), confirming previously published results (51, 52).

MAPK-stimulated Phosphorylation of Ser122 Is Required for Hypoxic Destabilization of Tal1 in Microvascular Endothelial Cells—To assess the importance of MAPK-mediated phosphorylation in hypoxic stimulation of Tal1 protein turnover, Tal1 protein expression was analyzed by Western blot analysis after exposing PD98059-pretreated MS1 cells to severe hypoxia. This MEK inhibitor increased the protein’s $t_{1/2}\text{initail}$, from 2 h to more than 6 h (Fig. 6A), consistent with the involvement of a MAPK in hypoxic acceleration of Tal1 turnover. To investigate the requirement of Ser122 phosphorylation specifically, cDNAs for Myc epitope-tagged wild-type protein and a Ser122 to Ala (S122A) mutant were transfected into MS1 cells, which were then made hypoxic. Whereas the level of Myc-tagged wild-type Tal1 declined with kinetics similar to that of the endogenously expressed protein, the S122A mutant was stable even in the face of severe hypoxia (Fig. 6B). Western blot and radioimmunoprecipitation analyses confirmed that the transfected pro-

FIG. 3. Tal1 degradation is mediated by the ubiquitin/proteasome pathway. A, Tal1-transfected HeLa cells were pretreated with the specific proteasomal inhibitor MG132 and cultured under normoxic or hypoxic (0% O2) conditions for 16 h. Tal1 expression was then measured by Western blot analysis. B, Tal1 protein abundance was determined by Western blot analysis in MS1 cells pretreated with MG132 (potent proteasome inhibitor), E64 (lysosomal protease inhibitor), LLnL (weak proteasome inhibitor), or LLM (calcium-dependent protease inhibitor) and then subjected to hypoxia (0% O2) for 16 h. C, extracts of MG132-treated HeLa cells transfected with expression vectors for Tal1, HA-ubiquitin, or both were immunoprecipitated with Tal1 antibody, and the immune precipitates were subjected to Western blot analysis with HA antibody. D, MG132-treated MS1 cells transfected with an expression vector for HA-ubiquitin were exposed to severe hypoxia (0% O2) or continued normoxia for 4 h and then extracted with RIPA buffer. Extracts were immunoprecipitated with Tal1 antibody, and immune precipitates were subjected to Western blot analysis with HA antibody.

FIG. 4. MG132 inhibits Tal1 protein turnover in hypoxic MS1 cells. MS1 cells were incubated for 16 h with 10 μM MG132 or vehicle, treated with 100 μM cycloheximide to inhibit further protein synthesis, and subjected to severe hypoxia (0% O2) or continued normoxia for 3, 6, or 9 h. Nuclear extracts were then fractionated on a 10% SDS-polyacrylamide gel and electrotransferred to a membrane. Tal1 protein was detected by Western blot analysis and quantitated from densitometry of the x-ray film. The mean percentage ± S.E. of Tal1 remaining is expressed relative to the time following the addition of the protein synthesis inhibitor. Data represent the results of three independent experiments.

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**FIG. 5.** Hypoxia elicits MAPK-mediated phosphorylation of Tal1 on Ser^{122}. MS1 cells metabolically labeled with [^{32}P]orthophosphate were preincubated with 50 μM of MEK inhibitor PD98059 or vehicle and then exposed to severe hypoxia (0% O_2) or continued normoxia for 2 h. A, radiolabeled Tal1 protein was immunoprecipitated from nuclear extracts with Tal1 antibody, fractionated on a 10% SDS-polyacrylamide gel, and analyzed by autoradiography. B, radiolabeled Tal1 protein was immunoprecipitated from nuclear extracts, fractionated on a 10% SDS-polyacrylamide gel, and transferred to a polyvinylidene difluoride membrane. Eluates were exhaustively digested with trypsin, and the resulting proteolytic products were subjected to twodimensional electrophoresis. Extracts of EGF-treated, Tal1-transfected COS-7 cells were processed in parallel as a positive control for Ser^{122} phosphorylation.

proteins were expressed at comparable levels before hypoxic exposure and that phosphorylation of the S122A mutant was reduced in cells made hypoxic compared with tagged wild-type protein (Fig. 6C). Together, these results demonstrate that MAPK-mediated phosphorylation of Ser^{122} is required for hypoxic destabilization of Tal1 in microvascular endothelial cells.

**DISCUSSION**

Hypoxia poses a significant challenge to cellular and organismal function and is an important stimulus of angiogenesis in developmental, physiological, and pathological contexts. As for other environmental stimuli (61), hypoxia induces the expression of a large number of genes that ultimately mediate the cell’s adaptations (62). This genomic response has increasingly been recognized to result from posttranslational modification (ubiquitination, most prominently) of specific transcription factors. Indeed, the levels of the most important transcriptional mediator of hypoxia-induced angiogenesis, HIF-1α, have been shown to be tightly controlled by this process (reviewed in Ref. 63). Although the consequence (proteasomal degradation) is identical, the oxygen tensions under which HIF-1α and Tal1 ubiquitination are elicited differ considerably.

Hypoxia also stimulates the phosphorylation of transcription factors. There is evidence of hypoxic activation of MAPKs for diverse cell types (45, 46, 48), including microvascular endothelial cells (51, 52), and MAPK-mediated phosphorylation of Elk-1 was shown to be important for hypoxic stimulation of c-fos and Egr-1 transcription (46, 48). HIF-1α has also been reported to be phosphorylated by MAPK, with an increase in its transcriptional potency but, interestingly, not its stability (51,
The ubiquitination and proteolysis of other transcription factors, including IκB (65), p53 (66), MyoD (67), c-Myb (68), progesterone receptor (69), microphthalmia (Mi) (70), BCL-6 (71), and Spi-B (72), are elicited by phosphorylation, although examples to the contrary have also been described (73, 74). Although ubiquitin-mediated destruction of BCL-6 (71) and Mi (70) by MAPK or MAPK-activated ribosomal S6 kinase 1 has recently been described, to our knowledge, this work establishes the first connection between hypoxic activation of MAPK and degradation of a transcription factor.

A separate mechanism has been described for the hypoxia-stimulated destruction of the transcription factor cAMP-response element-binding protein involving its decreased dephosphorylation by the serine/threonine phosphatase PP1 (75). Although a contribution of reduced dephosphorylation to hypoxic acceleration of TAL1 turnover cannot be completely excluded, the amino acid sequence encompassing Ser122 shows no homology to the proteasomal targeting motif in cAMP-response element-binding protein, PP1 at least would not be expected to act as a MAPK phosphatase, and, most directly, studies using a protein kinase inhibitor suggest that TAL1 stability is regulated at the level of phosphorylation.

The model most compatible with our results, then, has phosphorylation of Ser122 by a MAPK(s) acting to stimulate TAL1 ubiquitination and, ultimately, its trafficking to the 26 S proteasome. A critical step in this process must be in the conjugation of ubiquitin to TAL1, and we surmise that phosphorylation of this serine, which is sufficient to alter the protein’s mobility in denaturing polyacrylamide gels (53), must make it a more favorable substrate for an E2 ubiquitin-conjugating enzyme and/or E3 ubiquitin ligase. The observation that the N-terminal half of TAL1 encompassing Ser122 otherwise acts to stabilize the protein is demonstrated by the much shorter half-life in cells of the smaller pp24TAL1 isoform (76, 77) as compared with the full-length protein (data not shown).

Whereas MAPK-stimulated Ser122 phosphorylation is necessary for induced degradation of TAL1 in microvascular endothelial cells, in contrast to BCL-6 (71) and Mi (70), it is not sufficient to accelerate the protein’s turnover in heterologous cell types. Specifically, TAL1 protein stability was unaltered in...
hypoxic HeLa cells, in which MAPK can be presumed to have been activated (46) (Fig. 3B), and in EGF-treated Tal1-transfected COS-7 cells, in which MAPK-mediated Ser122 phosphorylation has been demonstrated directly (53). Thus, an additional factor(s), potentially a unique E2 and/or E3 enzyme, must be present in hypoxic endothelial cells for Ser122 phosphorylation to signal for increased ubiquitination. Proximity to a PEST domain, a proline-, glutamine-, serine-, and threonine-rich motif that acts to target proteins for degradation, is likely not involved, however, because no such regions are predicted to be present in either Tal1 or Mi (data not shown).

Activation from phosphorylation or, in the case of certain nuclear steroid receptors, ligand binding has recently been recognized to hasten transcription factor destruction (reviewed in Ref. 78). Furthermore, tumor-specific mutations were found to reduce the ubiquitination and subsequent proteasomal turnover of viral and cellular Myc proteins by disrupting critical phosphorylation sites (79–81). These and other studies (82, 83) have led to the concept that transcriptional activation domains also function as degradation motifs (“degrons”). In contrast to Mi, however, in which recruitment of the coactivator p300 (84)

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