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Biochemistry

$pVHL_{19}$ is a biologically active product of the von Hippel–Lindau gene arising from internal translation initiation

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ABSTRACT **The von Hippel–Lindau (VHL) gene encodes a protein consisting of 213 amino acid residues with an apparent molecular mass of 30 kDa ($pVHL_{30}$). Here we show that cells also produce a VHL protein ($pVHL_{19}$) that appears to arise as a result of internal translation from the second methionine within the VHL ORF. $pVHL_{30}$ resides primarily in the cytosol, with less amounts found in the nucleus or associated with cell membranes. In contrast $pVHL_{19}$, in biochemical fractionation experiments, is equally distributed between the nucleus and cytosol and is not found in association with membranes. $pVHL_{19}$, like $pVHL_{30}$, can bind to elongin B, elongin C, and Hs-Cul2 in coimmunoprecipitation assays and can inhibit the production of hypoxia-inducible proteins such as vascular endothelial growth factor (VEGF) and GLUT1 when reintroduced into renal carcinoma cells that lack a wild-type VHL allele. Thus, cells contain two biologically active VHL gene products.**

von Hippel–Lindau (VHL) disease is a hereditary cancer syndrome (1). Affected individuals are at high risk of developing multiple hypervascular tumors including retinal and central nervous system hemangioblastomas, clear cell renal cell carcinomas, pheochromocytomas, and pancreatic islet cell tumors (2).

The VHL susceptibility gene (3) is a tumor suppressor gene. Germ-line mutations affecting this gene have been documented in 80% of VHL patients (2). Tumor development in VHL patients is associated with loss or mutation of the remaining wild-type allele (4–6). Functional inactivation of both VHL alleles also occurs in the majority of sporadic renal cell carcinomas and cerebellar hemangioblastomas (4, 7–9). Restoration of VHL function in VHL(−/−) renal carcinoma cell lines inhibits their ability to form tumors in nude mice (10, 11).

We previously showed that the human VHL gene encodes an ≈30-kDa protein consisting of 213 amino acid residues ($pVHL_{30}$) (10). $pVHL_{30}$ resides primarily in the cytoplasm and to a lesser extent in the nucleus and in association with cell membranes (10, 12–14). Under certain experimental conditions, pVHL may shuttle between the cytoplasm and nucleus (15). A frequently mutated region of $pVHL_{30}$ binds to elongins B, C and Hs-Cul2 (14, 16–19). Elongins B and C were initially identified as factors that, when bound to elongin A, generate a complex (SIII) that promotes transcriptional elongation *in vitro* (16). Whether pVHL, elongin B, or elongin C play a role in transcriptional elongation *in vivo* is not currently known. In this regard, a significant amount of elongin B and C is present in the cytoplasm, suggesting that elongin B and C may perform additional functions unrelated to transcription (J. Conaway, R. Conaway, and W.G.K., unpublished data). Furthermore, elongin C and Hs-Cul2 are similar to Skp1 and Cdc53 in *Saccharomyces cerevisiae* (19, 21, 22). These latter proteins form multiprotein complexes that target certain proteins for ubiquitin-dependent proteolysis. Therefore, cytoplasmic elongin B and C, when bound to pVHL and Cul2, may affect the ubiquitination of as yet unknown target proteins.

VHL-associated neoplasms are hypervascular and overproduce angiogenic peptides such as vascular endothelial growth factor (VEGF) (23, 24). This result can now be understood in light of the recent finding that $pVHL_{30}$ inhibits the accumulation of hypoxia-inducible mRNAs. Among these are mRNAs that encode angiogenic peptides (such as VEGF), mitogenic factors (platelet-derived growth factor-B, transforming growth factor α), and proteins involved in cell metabolism such as the GLUT1 glucose transporter (11, 25–28). This effect, which has been genetically linked to the ability of pVHL to bind to elongins B/C and Cul2 (19), is mediated largely at the level of mRNA stability (11, 26).

In model systems inhibition of VEGF is sufficient to inhibit tumorigenesis (29). It is therefore conceivable that the ability of $pVHL_{30}$ to suppress tumor formation is linked to its ability to negatively regulate hypoxia-inducible proteins. In addition, $pVHL_{30}$ is also required for the proper assembly of an extracellular fibronectin matrix (30). Reorganization of extracellular matrix has been shown to influence tumor neovascularization and metastatic potential and thus could also contribute to tumor suppression by pVHL (31–33).

In previous experiments we detected multiple proteins, in addition to $pVHL_{30}$, that reacted specifically with monoclonal and polyclonal anti-VHL antibodies in both immunoprecipitation and immunoblot assays (10, 18). This result led us to ask whether cells contained VHL gene protein products in addition to $pVHL_{30}$. Here we show that the VHL gene encodes a second biologically active isoform, corresponding to amino acid residues 54–213 of $pVHL_{30}$, that migrates with an apparent molecular mass of 19 kDa (hereafter referred to as $pVHL_{19}$).

MATERIALS AND METHODS

Cell Culture. The 293 human embryonic kidney cell line and the 786-O renal carcinoma cell line, obtained from the American Type Culture Collection, were grown in DMEM containing 10% fetal clone (HyClone). 786-O cells stably transfected with pRc-CMV or pRc-CMV-HA-VHL(1–213) have been described (10, 19). 786-O cells stably transfected with pRc-CMV-HA-VHL(54–213) were generated in an identical fashion. Clonality was confirmed by immunofluorescence. Stable transfectants were grown in DMEM containing 10% fetal clone supplemented with 1 mg/ml G418. All cells were grown at 37°C in a humidified 10% CO_2-containing atmosphere.

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Abbreviations: VHL, von Hippel–Lindau; VEGF, vascular endothelial growth factor.
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Steady-state radioisotopic labeling was performed by methionine starvation for 1 h followed by growth in methionine-free DMEM supplemented with with [^{35}S]methionine [0.5 mCi/ml of medium (1 Ci = 37 GBq); EXPRE^{35}S^{35}S protein labeling mix (New England Nuclear)] and 2% dialyzed fetal bovine serum for 4 h. For pulse-chase analysis, cells were grown in the same labeling mix for 30 min without prior starvation. The cells were then washed twice with DMEM containing 10% fetal clone and placed in DMEM containing 10% fetal clone and 3 mg/ml L-methionine for the indicated periods of time. Cells were lysed in EBC lysis buffer (50 mM Tris, pH 8.0/120 mM NaCl/0.5% Nonidet P-40) containing 2 μg/ml aprotinin, 50 μg/ml phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 100 mM Na fluoride, and 200 μM Na orthovanadate. Lysates were clarified by centrifugation at 14,000 $\times$ *g* before use in immunoprecipitation reactions.

Transfections were performed as described by using the calcium-phosphate method (34).

Plasmids. pSP72-VHL(BZ3–4), which contains a cDNA spanning the entire VHL ORF (residues 1–213), has been described (10). A VHL cDNA encoding residues 54–213 was generated by PCR amplification of pSP72-VHL(BZ3–4) with primers 5′-GCGCGGATCCGCCACCATGGAGGCCGGG-CGGCCG-3′ and 5′-GAAGAATTCAATCTCCCATCCGT-TG-3′. This PCR product was restricted with *Bam*HI and *Eco*RI and used to replace the corresponding fragment of pSP72-VHL(BZ3–4) to make pSP72-VHL19 (LR 4-1). The authenticity of the PCR product was confirmed by DNA sequence analysis. The *Bam*HI–*Eco*RI VHL_{19} cDNA fragment from pSP72-VHL19 (LR 4-1) was subcloned into pcDNA-HA (35) to create pcDNA-HAVHL19 (LS 4-1) and into pSG5 (Stratagene) by using a three-way ligation to make pSG-VHL19 (LU 4-1). The *Hin*dIII–*Xba*I cDNA fragment from pcDNA-HAVHL19 (LS 4-1) was ligated into pRC/CMV (Invitrogen) to create pRC/CMV-HAVHL19 (LT 4-1).

Antibodies. Monoclonal anti-HA antibody was purchased from Boeringher Mannheim. The polyclonal anti-GLUT1 antibody GT-11A was purchased from Alpha Diagnostics (San Antonio, TX). The murine monoclonal anti-VHL antibody IG32 (18) and affinity purified rabbit polyclonal anti-VHL serum (R98) have been described (10). The anti-T antigen mAb pAB419 (36) was a gift of E. Harlow.

Immunoprecipitation and Immunoblotting. Immunoprecipitations were performed as described previously with $\approx$1 μg of the indicated mAb or 4 μl of the indicated antiserum. Immunoprecipitates were washed five times in NETN-900 buffer (20 mM Tris, pH 8.0/900 mM NaCl/1 mM EDTA/0.5% Nonidet P-40), boiled in SDS-containing sample buffer (0.0625 M Tris, pH 6.8/2% SDS/100 mM DTT/10% glycine/0.01% bromophenol blue), and resolved by SDS/PAGE. For immunoblot analysis, proteins were resolved by SDS/PAGE, transferred to poly(vinylidene difluoride) membranes (Bio-Rad), and incubated with affinity-purified R98 polyclonal anti-VHL antibody at 1:500 dilution, polyclonal anti-GLUT1 antibody at 1:50 dilution, or anti-HA antibody at 1:50 dilution. Bound antibody was detected colorimetrically by using an alkaline phosphatase-conjugated secondary antibody. For analysis of whole-cell extracts, protein loading was normalized after the determination of protein concentrations by the Bradford method.

***In Vitro* Translation.** Coupled *in vitro* transcription and translation were performed with TNT reticulocyte lysate (Promega) according to the manufacturer's instructions.

VEGF ELISA. Equal numbers of 786-O cells ectopically producing pVHL(1–213), pVHL(54–213), or stably transfected with the backbone expression plasmid (pRc/CMV) were plated on plastic dishes. Approximately 48 h later, when the cells had reached 70–80 confluence, the medium was changed. At the indicated time points, the concentration of VEGF in tissue culture supernatants was determined as described (26) by using a commercially available kit according to the manufacturer's instructions (R&D Systems). At the end of the assay cell extracts were prepared and quantitated, in duplicate, by using the Bradford method.

Partial Proteolytic Peptide Mapping. Partial proteolytic peptide mapping was performed precisely as described elsewhere (37).

Cell Fractionation. Cell fractionation was performed as described (38). Briefly, 293 cells were swollen for 30 min in hypotonic RBS buffer (10 mM Hepes, pH 6.2/10 mM NaCl/1.5 mM $MgCl_2$) containing protease inhibitors (2 μg/ml aprotinin, 50 μg/ml phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 100 mM Na fluoride, and 200 μM Na orthovanadate). Cells were disrupted by dounce homogenization by using a type B pestle. Less than 5% of the cells remained intact as determined by phase contrast microscopy. Nuclei were separated by centrifugation and washed twice in RBS with the above inhibitors. The cytoplasm was separated into cytosolic and membrane fractions by ultracentrifugation. The proteins contained in the nuclear and membrane fractions were extracted with EBC lysis buffer. The insoluble material after these EBC extractions was boiled in SDS-containing protein sample buffer before electrophoresis. Sufficient amounts of Tris (pH 8), NaCl, and Nonidet P-40 were added to the cytosolic fraction to render it equivalent to EBC. Immunoprecipitations of this cytosolic fraction, along with the soluble nuclear and membrane fractions, were performed as described above.

RESULTS

We previously detected cellular proteins that, despite recognition by multiple anti-VHL antibodies, migrated faster than $pVHL_{30}$ in SDS-polyacrylamide gels. The conceptual ORF of pVHL contains a second methionine at residue 54 (Met-54). The codon for this methionine is flanked by a purine at position -3 and a guanine at position $+1$ and thus might serve as a translation initiation site (39). To test this, 786-O VHL($-/-$) renal carcinoma cells were transiently transfected with a plasmid encoding VHL(54–213) (Fig. 1*A*, lanes 3 and 4) or with the empty vector (Fig. 1*A*, lanes 1 and 2). These cells, as well as 293 human embryonic kidney [VHL($+/+$)] cells, were metabolically labeled with [^{35}S]methionine, lysed, and immunoprecipitated with murine monoclonal anti-VHL (IG32) (lanes 2, 4, and 5) or control antibodies (lanes 1, 3, and 6). IG32 recognizes a pVHL epitope C terminal to Met-54 (data not shown). Bound proteins were resolved by SDS/PAGE and detected by immunoblotting with an affinity purified anti-VHL rabbit polyclonal antibody (R98). The anti-VHL mAb specifically immunoprecipitated two proteins of $\approx$30 and 19 kDa from the 293 cell extract (compare lanes 5 and 6). It has been shown previously that the $\approx$30-kDa species corresponds to pVHL residues 1–213 (10). The faster migrating species comigrated with the 19-kDa protein detected in 786-O cells transfected with the plasmid encoding VHL residues 54–213 (compare lanes 4 and 5). In a parallel experiment, these two comigrating proteins were detected autoradiographically, excised, and digested with *Staphylococcus aureus* V8 protease (Fig. 1*B*, *Left*) and α-chymotrypsin (Fig. 1*B*, *Right*). The partial proteolytic peptide maps of these two proteins (transfected and endogenous, respectively) and the *in vitro* translation product of a VHL(54–213) cDNA were identical (Fig. 1*B*). These results, taken together, suggest that the endogenous 19-kDa band identified in 293 cells is a pVHL isoform corresponding to amino acid residues 54–213. This pVHL19 isoform was also detected in ACHN and CAKI-1 renal carcinoma cell lines as well as the hepatoblastoma cell line Hep3B, all of which contain a wild-type VHL allele (data not shown).

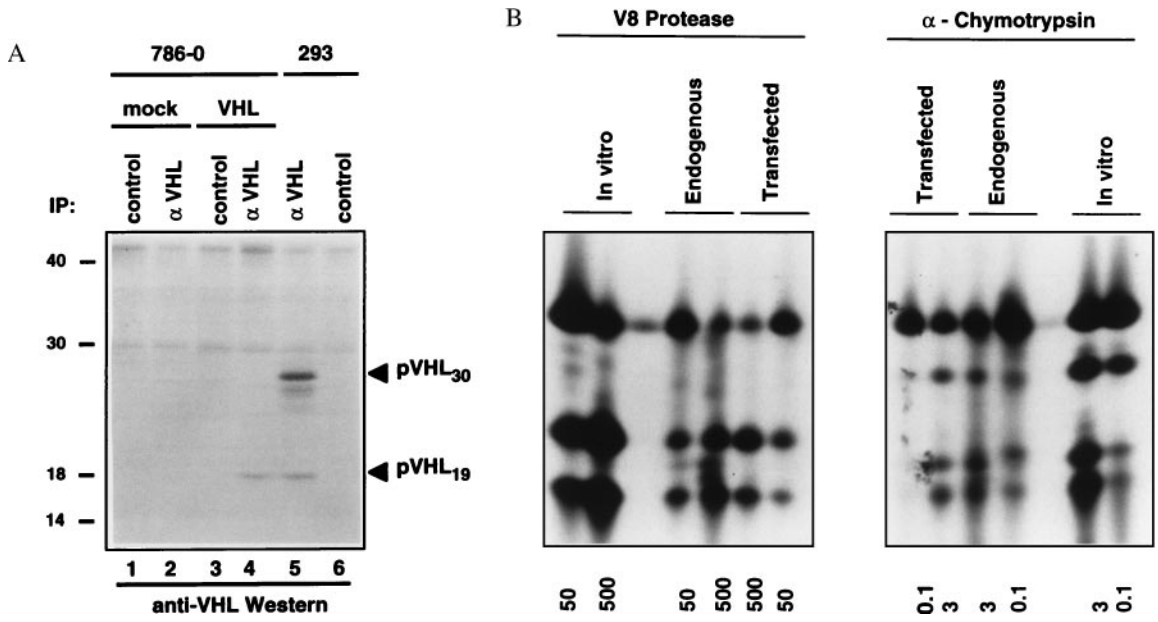

FIG. 1. Identification of $pVHL_{19}$ *in vivo*. (*A*) 786-O VHL(−/−) renal carcinoma cells were transfected with plasmid encoding $pVHL_{19}$ (lanes 3 and 4) or with the backbone expression plasmid (lines 1 and 2). The transfected cells and 293 human embryonic kidney cells (lines 5 and 6) were labeled with ^{35}S, lysed, and immunoprecipitated with control or IG32 monoclonal anti-VHL antibody as indicated. Bound proteins were detected by immunoblot analysis with affinity purified polyclonal anti-VHL antibody. (*B*) The ^{35}S-labeled $pVHL_{19}$ bands corresponding to lanes 4 and 5 of *A* were excised and digested with the indicated amounts of α-chymotryspin (micrograms) and V8 protease (nanograms). $pVHL_{19}$ translated *in vitro* was digested in parallel. Digestion products were resolved by SDS/PAGE and detected by autoradiography.

To ask whether $pVHL_{19}$ was a proteolytic fragment of $pVHL_{30}$, pulse-chase studies were performed (Fig. 2). $pVHL_{19}$ was present at the earliest time points and did not increase at the expense of $pVHL_{30}$. Indeed, the abundance of both $pVHL_{19}$ and $pVHL_{30}$ decreased with similar kinetics. Thus, $pVHL_{30}$ and $pVHL_{19}$ have similar half-lives and do not exhibit a precursor–product relationship.

To study the localization of endogenous $pVHL_{19}$, we fractionated asynchronously growing 293 cells (Fig. 3). Nuclear (N), membrane (M), and cytosolic (C) extracts were immunoprecipitated with anti-VHL antibody (Fig. 3, lanes 3–5). In parallel, a whole 293 cell extract (W) was similarly immunoprecipitated with anti-VHL (Fig. 3, lane 2) or a control antibody (Fig. 3, lane 1). Bound proteins were resolved by SDS/PAGE. In addition, the proteins remaining in the insoluble pellets during the preparation of the 293 whole-cell extract, membrane and nuclear fractions (Fig. 3, lanes 6, 7 and 10, respectively), were solubilized by boiling in SDS-containing running buffer and resolved in the same gel. As additional negative controls, insoluble pellets from membrane and nu-

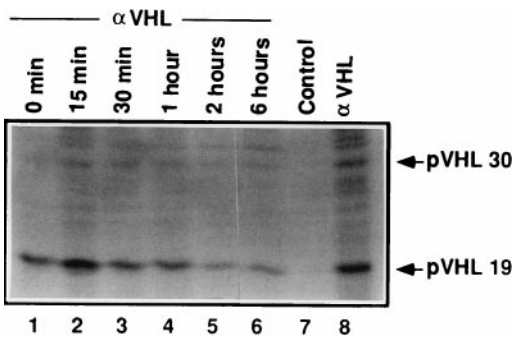

FIG. 2. Pulse-chase analysis of pVHL. 293 human embryonic kidney cells were pulse radiolabeled with [^{35}S]methionine. Cell extracts were prepared at the indicated time points after chase with unlabeled methionine and immunoprecipitated with an anti-VHL mAb (IG32) under antibody excess conditions (lanes 1–6). In parallel, 293 cells were radiolabeled with [^{35}S]methionine under steady-state conditions and immunoprecipitated with control (lane 7) or anti-VHL(IG32) (lane 8) antibody. Bound proteins were resolved by SDS/PAGE and detected by fluorography. Positions of $pVHL_{30}$ and $pVHL_{19}$, as confirmed by anti-VHL immunoblots performed in parallel, are shown by arrows.

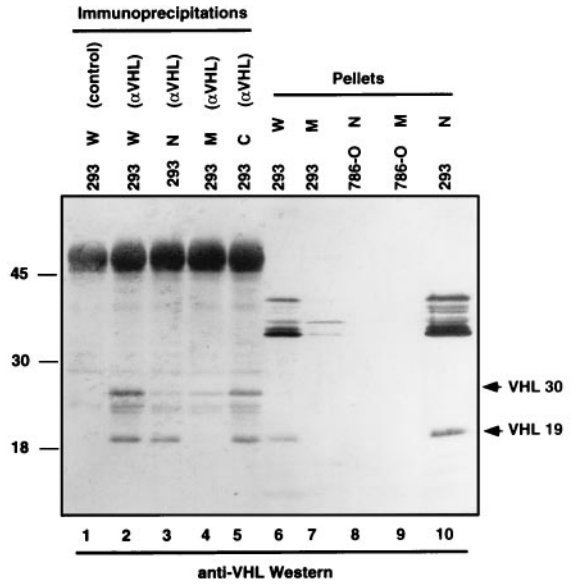

FIG. 3. Subcellular localization of $pVHL_{19}$. pVHL was immunoprecipitated with preimmune (lane 1) or affinity purified anti-VHL serum (lanes 2–5) from 293 whole-cell extracts (W, lanes 1 and 2) or the indicated cellular fractions [nuclei (N) (lane 3), membranes (M) (lane 4), and cytosol (C) (lane 5)]. The insoluble pellets following extraction of 293 cells (W) (lane 6), 293 nuclei (N) (lane 10), 293 cell membranes (M) (lane 7), as well as pellets from the corresponding insoluble pellets from 786-O VHL(−/−) cells [(N) (lane 8) and (M) (lane 9)] were boiled in SDS-containing protein running buffer. Immunoprecipitated (lanes 1–5) and resolubilized (lanes 6–10) proteins were resolved by SDS/PAGE and detected by immunoblot analysis with R98 anti-VHL antibody. Bound antibody was detected colorimetrically. The identities of the higher molecular weight species in lanes 6, 7, and 10 are unknown.

clear fractions derived from VHL(−/−) 786-O renal carcinoma cells were similarly prepared and analyzed in parallel (Fig. 3, lanes 8 and 9). After electrophoresis, proteins were detected by immunoblot analysis with an affinity purified anti-VHL polyclonal antibody. In keeping with earlier reports, the majority of $pVHL_{30}$ was detected in the cytosolic fraction and to a lesser extent in the membrane and nuclear fractions (compare lanes 3–5). In contrast, $pVHL_{19}$ reproducibly was found to equally distribute between the nuclear and cytosolic fractions and was not detected in the membrane fraction. Furthermore, in contrast to $pVHL_{30}$, a significant amount of $pVHL_{19}$ remained in the insoluble pellets following Nonidet P-40 extraction of whole cells or nuclei (lanes 6 and 10, respectively).

$pVHL_{30}$ has been shown previously to bind to elongin B, elongin C, HS-Cul2, and fibronectin. To ask whether this might be true for $pVHL_{19}$, 786-O subclones ectopically producing either HA-$pVHL_{30}$, or HA-$pVHL_{19}$ were metabolically labeled with [^{35}S]methionine and lysed. 786–0 [VHL(−/−)] empty vector transfectants were similarly prepared in parallel as additional negative controls. Lysates were immunoprecipitated with an anti-HA antibody (Fig. 4, lanes 2, 4 and 6) or a control antibody (Fig. 4, lanes 1, 3, and 5). Bound proteins were resolved by SDS/PAGE and detected autoradiographically. In keeping with earlier reports, elongin B, elongin C, and Hs-Cul2 coimmunoprecipitated with ectopically produced $pVHL_{30}$ (lane 4). $pVHL_{19}$ also bound to elongin B, elongin C, and Cul2 in this assay. Of note, the autoradiographic intensity of elongins B and C was considerably higher when coimmunoprecipitated with $pVHL_{30}$ than with pVHL19. Unlike $pVHL_{30}$, $pVHL_{19}$ did not bind to fibronectin (compare lanes 4 and 6).

$pVHL_{30}$ negatively regulates the expression of hypoxia-inducible proteins under normoxic (21% ambient oxygen tension) conditions. To examine the effect of $pVHL_{19}$ on the expression of such proteins, we generated multiple independent 786-O subclones that stably produce HA-$pVHL_{19}$. Three such clones (P1-5, P3-5, and P7-1), along with 786-O subclones expressing HA-$pVHL_{30}$ (D21 and G37) or empty vector transfectants (A6 and B2), were grown to 80% confluency under normoxic conditions. VEGF concentration in the tissue culture supernatant was measured by ELISA at 1, 2, and 6 h after medium change (Fig. 5*A*). At the end of this period cell extracts were prepared and immunoblotted with an anti-GLUT1 antibody (Fig. 5*B*, *Upper*) or anti-HA antibody (Fig. 5*B*, *Lower*). Inhibition of GLUT1 production and VEGF secretion by HA-$pVHL_{19}$ was comparable to that observed in cells producing HA-$pVHL_{30}$. This effect was previously shown to be specific as pVHL mutants that cannot bind to the elongin/Cul2 complex do not inhibit VEGF or GLUT1 production in these assays (19).

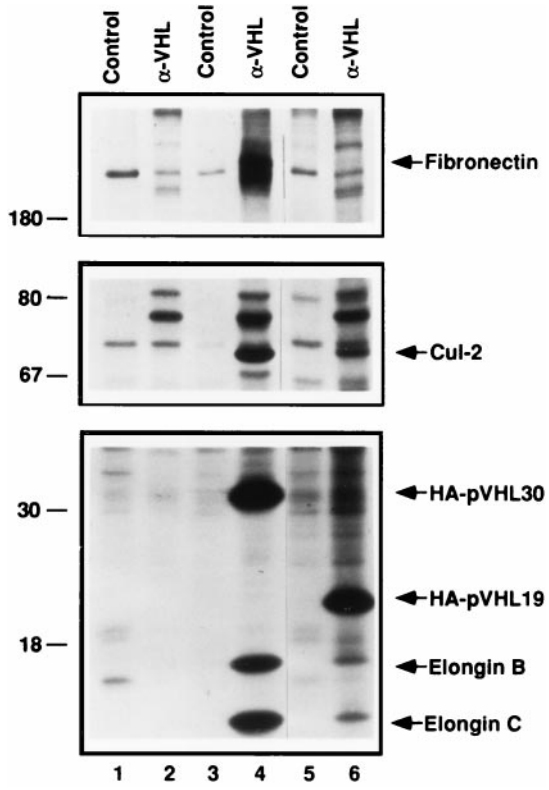

FIG. 4. $pVHL_{19}$-associated proteins. Stable 786-O renal carcinoma cell clones expressing HA-$pVHL_{30}$ (lanes 3 and 4), HA-$pVHL_{19}$ (lanes 5 and 6), or backbone vector transfectants (lanes 1 and 2) were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with control or anti-HA mAb, as indicated. Bound proteins were detected by autoradiography.

DISCUSSION

We identified an additional protein product of the VHL gene in cells. This protein, which migrates with an apparent molecular mass of 19 kDa, appears to correspond to pVHL residues 54–213 of the previously recognized VHL gene product ($pVHL_{30}$) as it comigrates with pVHL(54–213), and the partial proteolytic peptide maps of these two proteins are identical.

Only two VHL mRNAs have been detected in cells by Northern blot and RT-PCR analysis (7). One of these contains exons 1, 2, and 3 and encodes $pVHL_{30}$. The second, in which exon 1 is fused directly to exon 3, has not been shown to encode a protein. Furthermore, some renal carcinoma cell lines produce only the alternatively spliced mRNA (7), suggesting that its protein product, if made, is defective for pVHL tumor-suppression function. Both Met-1 and Met-54 are encoded by exon 1. Thus, it is unlikely that $pVHL_{19}$ arises because of alternative mRNA splicing. Third, pulse-chase experiments do not demonstrate a precursor–product relationship between $pVHL_{30}$ and $pVHL_{19}$. Thus, it is unlikely that $pVHL_{19}$ is a proteolytic fragment of $pVHL_{30}$. However, pVHL amino acid residue 54 is the second methionine in the VHL conceptual ORF. Furthermore, the corresponding ATG might serve as a translation site based on the presence of a purine at −3 and guanine at +1 (39, 40). The only remaining pVHL methionine is located at residue 211. Therefore $pVHL_{19}$ is most likely generated by internal translation initiation at Met-54. Similar conclusions have been reached by others working independently and in parallel to us (Robert Burk, personal communication; Nikolai Kley, personal communication).

It should be noted that our earlier studies of $pVHL_{30}$ biological functions were performed with plasmids that incorporated strong translation initiation sequences to direct the synthesis of the full-length protein (with or without an N-terminal epitope tag). These plasmids do not give rise to measurable levels of $pVHL_{19}$ as determined by immunoprecipitation and immunoblot assays (10, 18).

$pVHL_{19}$ and $pVHL_{30}$ appear to differ in their subcellular localization. First, unlike $pVHL_{30}$, $pVHL_{19}$ does not associate with cell membranes after biochemical fractionation. This result may account for its failure to coimmunoprecipitate with fibronectin as the interaction of $pVHL_{30}$ and fibronectin is restricted to this cellular compartment (30). Second, whereas $pVHL_{30}$ is primarily cytosolic, $pVHL_{19}$ partitions equally between the cytosolic and soluble nuclear fractions. Finally, unlike $pVHL_{30}$, a significant amount of $pVHL_{19}$ is found in the insoluble nuclear pellet following Nonidet P-40 extraction. This might suggest that a fraction of $pVHL_{19}$ is associated with structures such as the nuclear matrix.

A growing number of viral and cellular mRNAs are known to yield protein products as a result of internal translation inititation (41). There is precedent for the use of internal

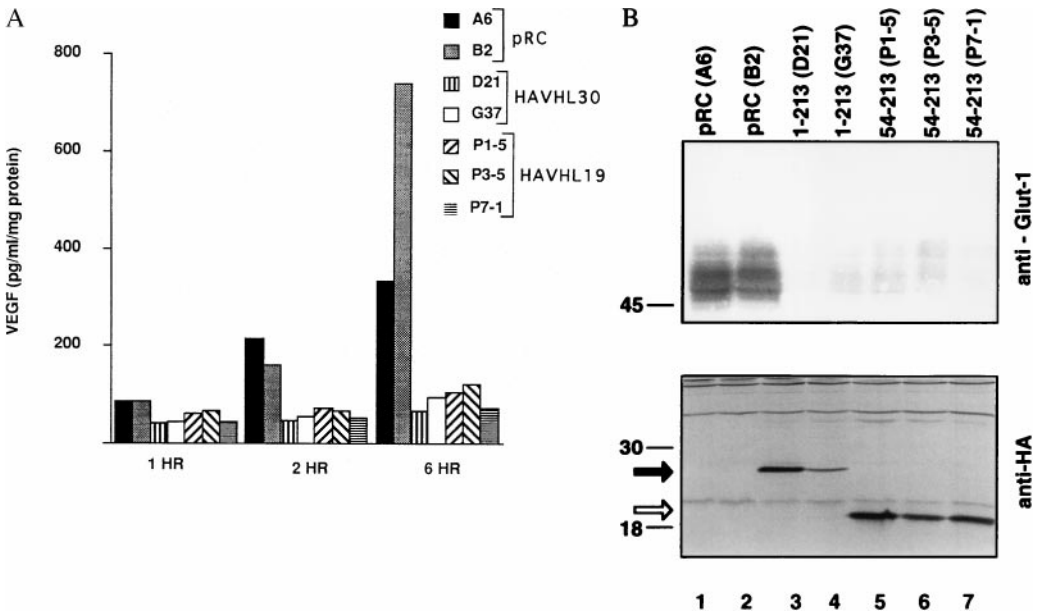

FIG. 5. Negative regulation of hypoxia-inducible proteins by $pVHL_{19}$. (*A*) Concentration of VEGF in the supernatant of the 786-O VHL(−/−) renal carcinoma cell clones ectopically producing HA-$pVHL_{30}$, HA-$pVHL_{19}$, or backbone vector transfectants (pRC) 1, 2 and 6 h after medium change. VEGF concentrations (pg/ml) were normalized to total cellular protein. (*B*) Whole-cell extracts (50 μg per lane) prepared from the above cells were resolved by SDS/PAGE and immunoblotted with anti-GLUT1 polyclonal (*Upper*) or anti-HA monoclonal (*Lower*) antibodies. Arrows indicate ectopically produced VHL proteins.

translation as a means of generating a protein product with a different subcellular localization than the corresponding full-length protein. For example, the full-length product of the *int-2* gene is nuclear whereas an internal translation product of this gene is secreted (42). Similarly, the full-length product of the MOD5 gene is mitochondrial, whereas an internal translation product is cytosolic (43). Furthermore, there are precedents for internal translation having a qualitative or quantitative effect on protein function (44, 45).

$pVHL_{19}$ and $pVHL_{30}$ share a short colinear sequence, corresponding to residues 157–172 of $pVHL_{30}$, that is necessary and sufficient for binding to elongins B and C *in vitro* (18, 46). Despite this, less elongin B and C coimmunoprecipitated with $pVHL_{19}$, as determined by autoradiography, than with $pVHL_{30}$. Several nonmutually exclusive possibilities can be envisioned. The first is that this reflects the differential subcellular localization of $pVHL_{19}$ and $pVHL_{30}$. In this regard, mixing experiments suggest that pVHL–elongin complexes do not form after cell lysis under the conditions employed here, and fractionation experiments suggest that pVHL binds to cytoplasmic elongins B and C (data not show). Second, $pVHL_{19}$, perhaps because of protein folding, may bind to elongins B and C with lower affinity than does $pVHL_{30}$. Finally, given the potential role of pVHL in ubiquitination (14, 19), it is possible that elongins are unstable when bound to $pVHL_{19}$.

In keeping with its ability to bind to elongin B, elongin C, and Hs-Cul2, however, $pVHL_{19}$ can inhibit the accumulation of hypoxia-inducible proteins such as VEGF and GLUT1. Noteworthy in this regard is the observation that the similarity between human, rat, and mouse VHL genes is highest in the region corresponding to $pVHL_{19}$ (3, 20, 47). Furthermore, tumor-derived VHL gene mutations almost invariably map C terminally to Met-54 (2). Conceivably, this reflects a selection pressure to inactivate both $pVHL_{30}$ and $pVHL_{19}$. Specifically, VHL gene mutations that mapped N terminal to Met-54 would still, in theory, be compatible with the production of wild-type $pVHL_{19}$, which, as shown here, is biologically active.

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