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16. Observations at night were analyzed for errors associated with stratification, which shifts transport toward higher frequency turbulence and decouples air flow at the sensor from the forest. We corrected lack of response to high-frequency CO_2 fluctuations by computing, in each hourly interval, the error in the sensible heat flux (usually 4 to 10%) when the high-frequency temperature signal was filtered to simulate the effective bandpass of the CO_2 analyzer (*15*). We assessed errors due to decoupling by comparing NEE in stratified periods with data from well-coupled (windy) nighttime intervals (momentum flux $< -500\ cm^{-1}\ s^{-2}$), and with the sum of measurements (*14*) for belowground, stem, and leaf respiration. It appears that unstable temperature gradients usually maintain turbulent exchange between 0 and 30 m, and errors associated with decoupling are small: nighttime NEE may be underestimated in midsummer by 0 to 0.5 kg of carbon per hectare per hour, corresponding to a possible overestimate of the annual net uptake of 0 to 0.5 metric ton per hectare.
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Identification of the von Hippel–Lindau Disease Tumor Suppressor Gene

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A gene discovered by positional cloning has been identified as the von Hippel–Lindau (VHL) disease tumor suppressor gene. A restriction fragment encompassing the gene showed rearrangements in 28 of 221 VHL kindreds. Eighteen of these rearrangements were due to deletions in the candidate gene, including three large nonoverlapping deletions. Intragenic mutations were detected in cell lines derived from VHL patients and from sporadic renal cell carcinomas. The VHL gene is evolutionarily conserved and encodes two widely expressed transcripts of approximately 6 and 6.5 kilobases. The partial sequence of the inferred gene product shows no homology to other proteins, except for an acidic repeat domain found in the procyclic surface membrane glycoprotein of *Trypanosoma brucei*.

Von Hippel–Lindau (VHL) disease is a familial cancer syndrome that is dominantly inherited and that predisposes affected individuals to a variety of tumors. The most frequent tumors are hemangioblastomas of the central nervous system and retina, renal cell carcinoma (RCC), and pheochromocytoma. The minimum birth incidence of VHL disease is one in 36,000, penetrance is almost complete by 65 years of age, and median actuarial life expectancy is reduced to 49 years, with RCC being the most common cause of death (*1*). Genetically, the disease gene behaves as a typical tumor suppressor (*2*) as defined in Knudson's theory of human carcinogenesis (*3*).

By positional cloning strategies, we and others have delineated the VHL gene region at human chromosome 3p25-p26 (*4*) and have obtained a nearly complete genomic coverage in overlapping yeast artificial chromosomes (YACs) and cosmid-phage contigs (*5*) (Fig. 1). In parallel with the cloning efforts, we established a physical map of the region by pulsed-field gel electrophoresis and began looking for gross rearrangements affecting the region. These efforts resulted in the discovery of nested constitutional deletions in three unrelated VHL patients (*6*). This finding and the availability of cloned DNA provided rapid access to the VHL gene. We reasoned that

the smallest of these three deletions should either encompass or interrupt the gene, and we identified a cosmid (cos11) mapping to the commonly deleted region (Fig. 1).

To isolate candidate genes, we searched the deleted region for transcribed sequences by screening cDNA libraries with probes representing evolutionarily conserved sequences in cos11. Two apparently unrelated cDNA species, each represented by overlapping clones, were isolated from a λgt11 teratocarcinoma cDNA library. The first, denoted g6, was detected by the telomeric end of cos11, and the second, denoted g7, by the cosmid's proximal end (Fig. 1).

To identify the VHL gene, we evaluated g6 and g7 by analyzing their expression in target tissues, determining their copy number, and searching for inactivating mutations that followed transmission of the disease. Such mutations are indicative of a tumor suppressor gene (*3, 7*). As there is no evidence for genetic heterogeneity in VHL disease (*1, 4*), the critical gene is thought to be encoded by a single chromosomal locus. We concluded that g6 was unlikely to be the VHL gene, because we detected no g6 mutations in 120 VHL patients and because we found an additional cross-hybridizing locus on the X chromosome.

By contrast, g7 proved to be a strong candidate for the VHL gene. We first studied the expression pattern of g7 by Northern (RNA) blotting (Fig. 2) (*8*). Transcripts were observed in all human tissues tested (*8*), including brain and kidney, tissues frequently affected in VHL disease. The transcripts were of two distinct sizes, 6 and 6.5 kb (Fig. 2), and were expressed in a tissue-specific and developmentally selective manner; the 6-kb RNA alone and the 6.5-kb RNA alone were expressed in fetal brain and fetal kidney, respectively, whereas both RNAs were expressed in adult tissues. The two transcripts may represent alternatively spliced forms of g7 mRNA.

We then demonstrated that the g7 cDNA defines a single copy gene. There were no differences between the restriction maps of genomic and cloned (cosmid-phage contig) g7 DNA, and fluorescence in situ hybridization (FISH) with g7 probes revealed only one locus on chromosome 3p25 (*9*). Furthermore, Southern (DNA) analysis showed that the g7 sequence is highly conserved across species as diverse as mammals, drosophila, and sea urchin (*10*). This extensive evolutionary conservation suggests that g7 encodes a fundamental cellular function (*11*), a hypothesis compatible with its putative role as a tumor suppressor (*7*).

Finally, we searched for inactivating mutations in constitutional DNA derived from 221 unrelated VHL patients, including eight patients classified as "new mutations," by Southern blot analysis with the 1.5-kb g7 cDNA probe. This probe detects a single invariant 20- to 22-kb Eco RI fragment in normal DNA, as determined by previous tests on >100 unrelated DNA samples provided by the Centre d'Etude du Polymorphisme Humain (CEPH). We found aberrant bands, ranging in size from 4 to 25 kb, in 28 of the 221 (12%) VHL patients (Fig. 3A). These rearrangements were confirmed with several other restriction enzyme digests (Bam HI, Bgl I, Bgl II, Dra I, Eco RV, Hind III, Pst I, and Pvu II) and were shown to follow transmission of the disease in VHL families, including a new mutation family (Fig. 3B).

Genomic probes flanking the Eco RI sites of the 20- to 22-kb fragment were then used to map the rearrangements. In 25 of 28 patients, the alterations were entirely confined to the invariant Eco RI fragment, whereas in the remaining three patients they also involved the adjacent telomeric 20-kb fragment (Fig. 1B). The most likely mechanism that would account for single aberrant bands originating from the invariant Eco RI fragment would be deletions or insertions within this fragment or deletions removing the flanking Eco RI sites.

To prove that the aberrant bands were due to deletions and to map them precisely, we used polymerase chain reaction (PCR)–generated subfragments representing the entire cDNA and genomic fragments from within the Eco RI fragment as probes in Southern blot analyses. The results un-

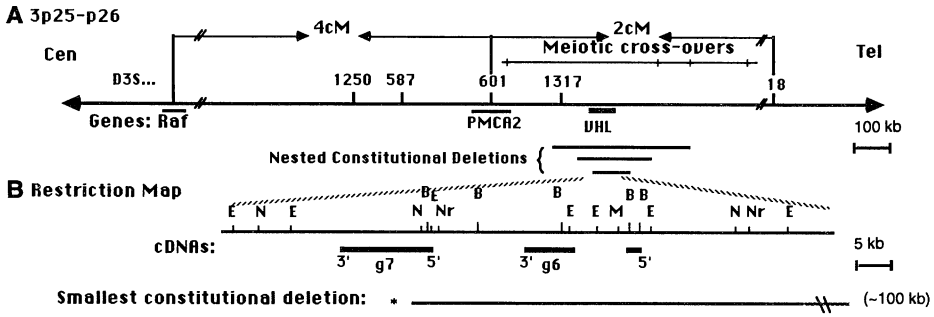

Fig. 1. (**A**) Genetic and physical map of the chromosome 3p region encompassing the VHL gene. Genetic and physical distances between selected markers are shown in centimorgans (cM) and kilobases, respectively. The VHL locus was positioned on the map by multipoint linkage analysis and meiotic mapping (*4, 18*). The locations of selected crossovers are indicated by short vertical lines, and the positions of the nested deletions discovered in the constitutional DNA of three unrelated VHL patients (*6*) are shown under the map. The genes are not drawn to scale. Cen, centromere; Tel, telomere. (**B**) Restriction map of cos3, cos11, and phage p191 detailing the positions of g6 and g7 cDNAs isolated by screening the λgt11 teratocarcinoma cDNA library with conserved fragments from cos11. The orientation of the cDNAs was established by sequencing and restriction mapping to the contig. The beginning of the smallest constitutional deletion is indicated by an asterisk and line. Restriction sites: B, Bam HI; E, Eco RI; N, Not I; Nr, Nru I; M, Mlu I.

Table 1. Germline (VHL) and somatic (sporadic RCC) mutations in the candidate VHL gene.

Patient	Mutation (nt number)	Effect
VHL family		
VA	8-nt insertion (714)	Frameshift
E	9-nt in-frame deletion (456)	Deletion of three amino acids
CS	3-nt in-frame deletion (434)	Deletion of one amino acid
Sporadic RCC		
UOK118	1-nt deletion (737)	Frameshift
UMRC5	1-nt deletion (737)	Frameshift
UMRC6	10-nt deletion (715)	Frameshift
A498	5-nt deletion (638)	Frameshift
UOK151	C → A transversion (761)	Stop codon

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equivocally demonstrated that 18 of the rearrangements were deletions, because only part of the cDNA failed to detect the novel band in each patient. The deletions could be classified into three groups. In three patients with relatively large (~15 kb) deletions extending into the telomeric adjacent fragment but not reaching the g6 locus (Fig. 1B), only the 5′ end of the cDNA fell within the deletions, and the remainder of the cDNA was retained. In another group of five patients, the deletions included most of the cDNA but not coding sequences at the 5′ end. In the remaining ten patients, the deletions appeared to remove only the 3′ untranslated portion of the cDNA. Clearly, the three deletions in the first group did not overlap with the deletions in the other patients. Because we have not yet defined the borders of the g7 gene, we do not know if any of these deletions are entirely intragenic. However, our finding that VHL patients harbor large, nonoverlapping deletions in g7 provides strong evidence that it is the VHL gene.

We searched for additional alterations by single-strand–conformational polymorphism (SSCP) analysis of DNA and reverse transcription–PCR (RT-PCR)–amplified cDNA from sporadic RCC cell lines and VHL lymphoblastoid cell lines. We chose to study RCC because Knudson's model (*3*) predicts that sporadic cancers should be associated with mutations in the same loci affected in the corresponding hereditary cancer. We identified aberrant patterns in five RCC cell lines and found that four of them were small [1- to 10-nucleotide (nt)] deletions that created frameshift mutations and, presumably, truncated proteins (Table 1). In the fifth RCC cell line, the change was a nonsense mutation, resulting from a C → A transversion at nt 761 (Table 1).

Through SSCP analysis, we also detected aberrant patterns in VHL patients and these patterns followed transmission of the disease (Table 1). One patient was found to have an 8-nt (TTGTCCGT) insertion at nt 714 (Fig. 3C). This insertion created a shift in the reading frame and presumably a truncated protein. Another patient had an in-frame 3-nt deletion at nt 434, predicted to remove Ile^{146} in the gene product. A third patient had an in-frame 9-nt deletion at nt 456, predicted to remove Arg^{153}-Val^{154}-Val^{155}. Together, these results strongly support the conclusion that g7 is the VHL and the sporadic RCC tumor suppressor gene.

The compiled sequence of the g7 cDNA clones revealed a short open reading frame (ORF) with a high probability (>95%) of being a protein coding sequence (*12*). This ORF encoded only 284 amino acids (Fig. 4), so the remainder of the g7 cDNA most likely represents the 3′ untranslated region of the gene. Neither the nucleotide nor the predicted amino acid sequences showed any significant homology to genes or proteins in the databases (*13*). We note, however, the presence of eight copies of an acidic tandemly repeated pentamer Gly-X-Glu-Glu-X (residues 85 to 125) that is 48% identical with a pentamer (Gly-Pro-Glu-Glu-Pro) that is repeated seven times in the procyclic surface membrane protein of *Trypanosoma*

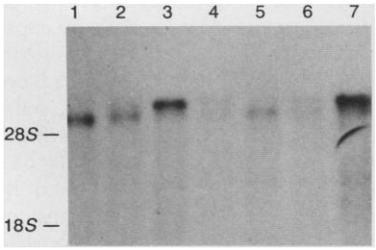

Fig. 2. Northern blot analysis of g7 gene expression in human fetal and adult tissues. The polyadenylated mRNA (1 μg per lane) was purchased from Clontech Laboratories, Inc. (Palo Alto, California). The probe was g7 cDNA. Lane 1, fetal brain; lane 2, adult brain; lane 3, fetal kidney; lane 4, adult kidney; lane 5, adult cerebellum; lane 6, adult adrenal; and lane 7, adult prostate. The sizes of the g7 transcripts were determined from the positions of 28*S* and 18*S* ribosomal RNAs.

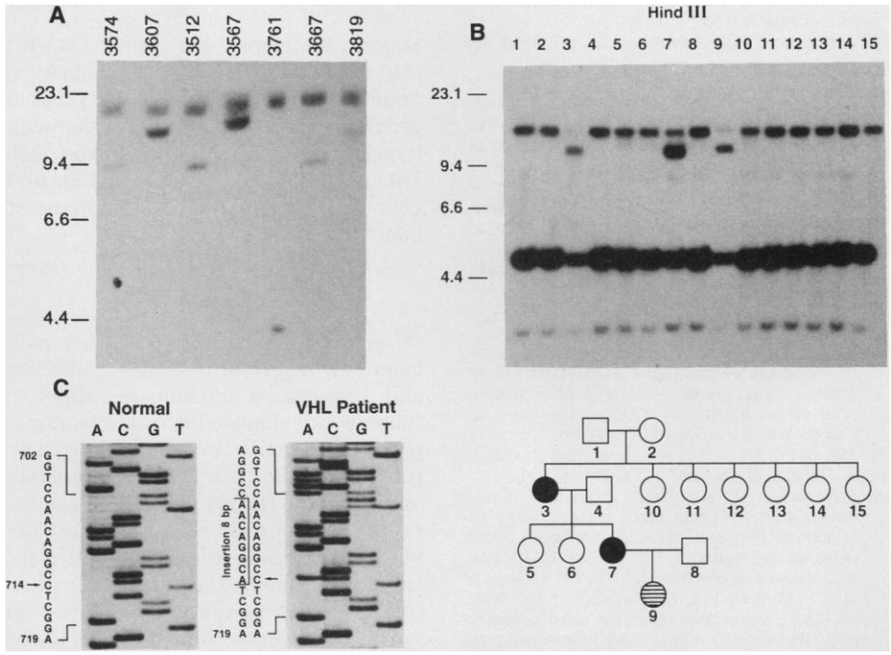

Fig. 3. Detection of rearrangement mutations is constitutional DNA of patients with VHL disease. The DNA samples were subjected to Southern blot analysis (*5*) with g7 cDNA as probe. (**A**) DNA from lymphoblastoid cell lines of seven unrelated VHL patients was digested with Eco RI. The normal invariant band is ~20 to 22 kb; the aberrant bands range in size from 4 to 25 kb and probably result from deletions. The patient code numbers are indicated above the lanes. (**B**) DNA from lymphoblastoid cell lines of members of a new mutation family (coded "X") was digested with Hind III. The pedigree shows the positions of affected individuals (filled circles) and of an individual who is predicted to be affected (hatched circle). Note that in this new mutation family the disease was transmitted to the next generation. (**C**) SSCP analysis (*19*) of genomic DNA from a VHL patient with an 8-nt insertion mutation at nt 714 (Table 1). The sequence shown is that of the antisense strand; therefore, the inserted nucleotides are 5′-ACGGACAA-3′.

Fig. 4. Partial amino acid sequence of the g7 protein, deduced from the cDNA sequence. The repeated acidic domain Gly-X-Glu-Glu-X is underlined. Sequence analysis (*5*) was performed on two overlapping clones of 1.54 and 1.65 kb isolated from the teratocarcinoma cDNA library and on an exon amplified by PCR. The 1817- nt sequence has been deposited with GenBank (accession number L15409). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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  1  PRLRYNSLRC WRILLRTRTA SGRLFPRARS ILYRARAKTT EVDSGARTQL
 51  RPASDPRIPR RPARVVWIAE GMPRRAENWD EAEVGAEEAG VEEYGPEEDG
101  GEESGAEESG PEESGPEELG AEEEMEAGRP RPVLRSVNSR EPSQVIFCNR
151  SPRVVLPVWL NFDGEPQPYP TLPPGTGRRI HSYRGHLWLF RDAGTHDGLL
201  VNQTELFVPS LNVDGQPIFA NITLPVYTLK ERCLQVVRSL VKPENYRRLD
251  IVRSLYEDLE DHPNVQKDLE RLTQERIAHQ RMGD
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brucei (*14*). This protein belongs to a novel class of glycan-anchored membrane proteins (*15*) that are thought to function in signal transduction (*16*) and intracellular targeting (*17*). The presence of this acidic repeat domain suggests that the VHL protein may be localized on the cell membrane and may be involved in signal transduction or cell adhesion. Further clues to the function of the VHL protein may emerge as more sequence information is obtained.

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8. Polyadenylated RNA from all tissue types tested (heart, skeletal muscle, brain, placenta, lung, liver, kidney, pancreas, prostate) showed a strong hybridization signal at 6 to 6.5 kb. In addition, expression was observed by RT-PCR analysis of total RNA from VHL lymphoblastoid cell lines. RNA and corresponding cDNA were prepared and amplified (*5*) with primers representing the g7 cDNA sequence (Fig. 4).
9. FISH was performed as in K. Tory *et al.* [*Genomics* **13**, 275 (1992)]. Phage p191, containing an 80-kb insert, was isolated by D. A. Smoller from a three-hit p1 phage genomic library (Genome Systems, Inc., St. Louis, MO).
10. The Southern blots contained DNA samples (purchased from BIOS Laboratories, New Haven, CT) from the following species: human, chimpanzee, macaque, cow, rat, mouse, chicken, frog, fruit fly, sea urchin, and yeast (*Saccharomyces cerevisiae*). Blots were (pre)hybridized in Church buffer [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991 (1984)] at 65°C for 18 hours and washed in 0.1× Church buffer at 60°C for 60 min.
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20. This work is the result of a 6-year effort and represents a truly communal enterprise. We appreciate the cooperation of the VHL families in the United States, Canada, and Europe. B. Zbar, M. Lerman, and the NCI–FCRDC group are grateful to A. Rabson for his unwavering support. We thank M. F. Singer for the λgt11 teratocarcinoma cDNA library, H. B. Grossman for RCC cell lines UMRC5 and UMRC6, E. Brundage for help in screening YAC libraries, and J. Klabansky and D. Shores for managing the manuscript. Supported by grants from Action Research and the Cancer Research Campaign (to E.R.M.), the Japanese Overseas Cancer Fellowship of the Foundation for Promotion of Cancer Research (to M.Y.), and the William K. Warren Foundation (to D.S.R.D.).

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Repression of MHC Class I Gene Promoter Activity by Two-Exon Tat of HIV

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Major histocompatibility complex (MHC) class I molecules are the major receptors for viral peptides and serve as targets for specific cytotoxic T lymphocytes. Human immunodeficiency virus–type 1 (HIV-1) specifically decreased activity of an MHC class I gene promoter up to 12-fold. Repression was effected by the HIV-1 Tat protein derived from a spliced viral transcript (two-exon Tat). These studies define an activity for two-exon Tat distinct from that of one-exon Tat and suggest a mechanism whereby HIV-1–infected cells might be able to avoid immune surveillance, allowing the virus to persist in the infected host.

Major histocompatibility complex molecules play a pivotal role in the initiation and propagation of immune responses. Immune surveillance for viral infections is provided primarily by MHC class I antigens, which bind intracellularly generated viral peptides and act as targets for antiviral cellular immune responses (*1*). Many viruses are known to repress MHC class I expression, among them a number of retroviruses such as the Moloney leukemia virus (*2*). Decreases in MHC class I levels provide a mechanism for the virus to evade the host immune response. HIV-1 is a complex retrovirus that primarily infects $CD4^+$ T cells and monocytes, ultimately causing a depletion in the $CD4^+$ T cell population and a profound immunodeficiency (*3*). Because HIV-1 is known to establish persistent infections, suggesting a viral mechanism to avoid immune surveillance, we have examined its effect on MHC class I expression.

To assess the effect of HIV-1 on MHC class I expression, we transiently transfected human HeLa cells with an HIV-1_{LAI}–derived construct, pNL-A1, which expresses all viral gene products except Gag and Pol (*4*), and measured cell surface class I expression by staining with an antibody to human leukocyte antigen, W6/32, 48 hours after transfection. To distinguish pNL-A1–transfected from nontransfected HeLa cells, we also stained cells with an antibody to gp120 (pNL-A1 encodes gp120). Two-color flow cytometry was performed, and the mean cell surface level of class I on $gp120^+$ cells was compared with that on $gp120^-$ cells in the same population (*5*). Whereas $gp120^-$ cells stained with a mean fluorescence intensity (MFI) of 100 ± 8, $gp120^+$ cells expressed significantly lower levels of MHC class I, with an MFI of 49.2 ± 2.1 ($P < 0.0004$, five independent experiments). Thus, one or more HIV-1 gene products (but not Gag or Pol) are capable of markedly decreasing endogenous MHC class I expression. The extent of reduction observed in the present studies reflects only the difference that occurs during the transient 48-hour assay and could be an underestimate of the actual reduction of class I expression occurring in transfected HeLa cells. These results are consistent with a previous report describing a transient decrease in class I expression in HIV-1–infected T cell lines (*6*). Changes in class I expression of the magnitude observed here have been shown to alter susceptibility of cells to cytolysis by cytotoxic T cells (*7*).

To determine whether the HIV-1–mediated reduction of class I expression was transcriptional, we examined the effect of

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