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VHL tumor suppressor complex binds hyperphosphorylated large subunit of RNA Polymerase II through a proline hydroxylation motif and targets it for ubiquitination.

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Abstract

RNA polymerase II is an enzymatic complex responsible for the regulated synthesis of mRNA. Transition from transcription initiation to elongation involves phosphorylation of the large subunit (Rpb1) of RNA polymerase II on the repetitive carboxy-terminal domain. Elongation competent, hyperphosphorylated Rpb1 is subject to extensive ubiquitination. Using computer modeling we identified that a region of Rpb1 and the adjacent subunit 6 of RNA polymerase II (Rpb6) share similarity in sequence and structure with the von Hippel-Lindau tumor suppressor (pVHL) binding domain of hypoxia-inducible factor 1α (HIF1á). pVHL-associated E3 ligase ubiquitinates HIFα, targeting it for proteasomal degradation. In agreement with the computational model, we show biochemical evidence that pVHL binds hyperphosphorylated Rpb1 in a proline-hydroxylation dependent manner, and that this interaction is augmented by UV stress. We also demonstrate that pVHL regulates constitutive and UV-induced ubiquitination of the hyperphosphorylated Rpb1 *in vivo.*

The pVHL tumor suppressor protein-associated complex containing elongin B, elongin C, cullin 2 and Rbx-1 (1-3) has been recognized as a primary ubiquitin ligase for ubiquitination of the α subunits of the hypoxia-inducible transcription factors (HIFs). Ubiquitination of HIFα subunits targets them for proteasomal degradation (4-6). During normoxia, HIF-αs are translated and the conserved proline residues P564 and P402 are hydroxylated by the O_2, Fe(II) and oxyglutarate-regulated Egl-9 family of prolyl hydroxylases (7-11). Hydroxylases recognize L(XY)LAP motifs, where the proline is subject to hydroxylation (12). During hypoxia, proline hydroxylation is inhibited, HIF-αs are not ubiqutinated, accumulate and regulate transcription of the HIF-responsive genes (7-10). Loss of pVHL function in the VHL disease leads to accumulation of HIF-αs during normoxic conditions causing constitutive induction of a number of HIF-responsive genes, including angiogenic vascular endothelial growth factor (VEGF) (13,14). This in turn, results in the formation of highly vascular tumors such as hemangioblastomas and angiomas, as well as highly vascular renal clear cell carcinomas (15). VHL disease is also associated with the pheochromocytomas, non-malignant tumors of adrenal medulla chromaffin cells, that produce and release large quantities of catecholamines (16,17). Recently, we presented *in vivo* evidence that pVHL regulates expression of the rate-limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase, at the level of transcriptional elongation in pheochromocytoma derived PC12 cells (18,19). Low levels of pVHL correlate with more efficient transcription of the full length TH transcripts (19), while high levels of pVHL result in the transcript elongation block between exon 6 and 8 of the TH gene (18). The presence of the elongation arrest site within this region of the TH gene has been confirmed using *in vitro* transcriptional analysis (20).

Processive elongation of the initiated transcripts involves reversible hyperphosphorylation of tandemly repeated heptapeptides within the carboxy-terminal domain (CTD) of Rpb1 within the

RNA polymerase II complex (21). This elongation-competent, hyperphosphorylated Rpb1 is regulated by ubiquitination in a transcription – dependent manner (23,24). In particular, ubiquitination of the hyperphosphorylated Rpb1 is induced by UV radiation and DNA damage (25-27) suggesting the role of Rpb1 ubiquitination in the transcription coupled repair (28). In yeast, this ubiquitination is mediated by a HECT-class Rsp5 ubiquitin ligase (29). The nature of the E3 ligase in mammalian cells is unknown. Here, we show computationally predicted similarity between pVHL binding domain of HIF1α and its plausible counterpart on Rpb1/Rpb6 units. In particular, the amino acid motif containing hydroxylated proline within HIF1α is conserved in Rpb1 (aa 1460-1465). We further show biochemical evidence that pVHL-associated protein complex interacts directly with the hyperphosphorylated Rpb1, leading to its ubiquitination in mammalian cells. Our data suggest that pVHL complex may function as an E3 ubiquitin ligase towards the Rpb1. The identified interaction may represent a general mechanism involved in regulation of gene transcription, and in pathogenesis of VHL disease-related tumors.