Tumor Suppression and Transcription Elongation: The Dire Consequences of Changing Partners

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The initiation of transcription has long been thought to be the predominant mechanism of regulating gene expression—to the extent that faulty gene expression in human diseases, including cancer, has been assumed to be due to the disruption of initiation. For example, the inappropriate expression of oncogenes caused by chromosomal translocations that juxtapose oncogenes and regulatory elements (*1*) has been attributed to alterations in transcription initiation. Similarly, neoplastic transformation associated with the mutation or deletion of regulatory factors (such as the retinoblastoma susceptibility protein Rb or p53) for genes controlling cell cycle progression, development, or cell death has been ascribed to defective initiation. However, this focus on initiation may be shortsighted; in fact, the subsequent step in transcription, elongation, has been recognized as an important control mechanism in prokaryotes for many years, and, in the past decade, the number of eukaryotic genes demonstrated to be regulated by elongation has increased steadily (*2*).

The importance of transcriptional elongation in regulating eukaryotic gene expression is emphasized by three exciting papers in this week's issue of *Science*. The work of Duan *et al.* (*3*), Aso *et al.* (*4*), and Kibel *et al.* (*5*) indicates that a transcription elongation factor called Elongin is negatively regulated by the product of the von Hippel–Lindau tumor suppressor gene (*VHL*) (Fig. 1). In patients with von Hippel–Lindau disease, *VHL* is defective; the consequent interference with elongation control mechanisms has drastic results. Individuals with germ-line mutations in the *VHL* gene (*6*) are predisposed to multiple forms of cancer including renal carcinoma, hemangioblastoma, and pheochromocytoma. That *VHL* is a key player in the genesis of at least some of these cancers is strengthened by the finding that mutations in this gene occur in nonhereditary (sporadic), as well as hereditary, forms of renal carcinoma (Fig. 2). The predicted sequence of the VHL protein, reported in 1993 (*6*), had not revealed any clues to its function.

RNA polymerase II pauses at several positions immediately downstream of the transcription initiation site (*7*). It encounters additional pause sites further downstream, and much of the time required for the synthesis of an RNA molecule is likely due to the idling of polymerase at pause sites along the gene. The frequency of release out of these pause sites would be an important regulatory step for transcription, and, indeed, transactivator proteins recruited to the promoter through enhancer, as well as factors that modify polymerase at sites within a gene, appear to influence polymerase pausing at sites both proximal to and distant from the promoter (*7*, *8*).

In bacteriophage lambda, the prokaryotic prototype for transcriptional regulation at the level of elongation, the phage-encoded proteins Q and N are required for the modification of the bacterial RNA polymerases into elongation-competent forms at promoter proximal and downstream regions (*9*). In eukaryotes, the *Drosophila* transcription factor P-TEF catalyzes the conversion of early, termination-prone transcription complexes into productive elongation complexes (*10*). In addition, two mechanistically distinct classes of transcription elongation factors have been described: (i) TFIIS, which facilitates elongation through "roadblocks" (DNA-bound proteins, attenuation sites) by cleavage of the nascent transcript to permit "backing up" of polymerase (*11*); and (ii) transcription elongation factors TFIIF and Elongin (originally named SIII) which increase the rate of elongation through suppression of RNA polymerase pausing (*12*).

The transcription factor Elongin was originally identified as an enzymatic activity (SIII) that increases the rate of RNA polymerase complex movement along the DNA template (*13*). Continuing their elegant work, Aso and co-workers (*4*) now demonstrate that Elongin A is the catalytic subunit of the Elongin complex, whereas

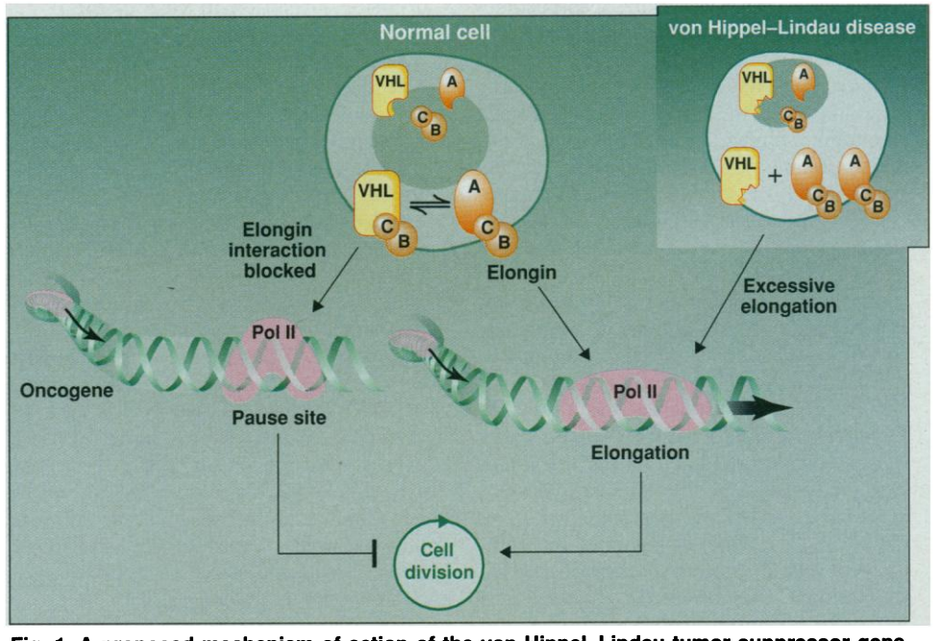

Fig. 1. A proposed mechanism of action of the von Hippel–Lindau tumor suppressor gene. Elongin is a heterotrimeric transcription factor, consisting of subunits A, B, and C. The von Hippel–Lindau tumor suppressor gene product VHL sequesters the positive regulatory subunits B and C, preventing their interaction with the catalytic subunit A. In the absence of Elongin, RNA polymerase pauses at several sites along the gene, leading to a low transcript level of as yet unidentified target genes. The binding of Elongin A to the B/C complex results in relief of pausing and a high transcript level. Thus, the ratio of VHL and Elongin A is a significant determinant of the level of target gene expression. Elongin may indirectly interact with RNA polymerase to prevent the strained conformation associated with pausing (see text). Mutations of the *VHL* gene result in a high level of Elongin A/B/C complex and in suppression of RNA polymerase II pausing. Several oncogenes, including the *c-myc* family and *c-fos*, which are positive regulators of cell division, are controlled at the level of transcript elongation. Thus, the neoplastic pathway of *VHL* mutation may involve the failure to regulate such target genes.

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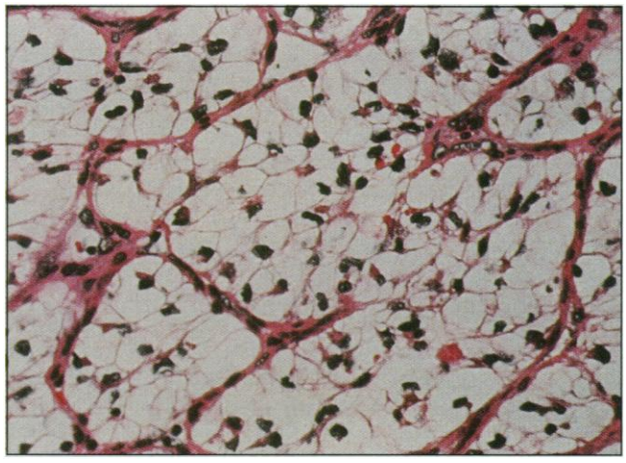

Fig. 2. Tumor from a patient with sporadic clear-cell renal carcinoma. The majority of these patients show functional loss of both *VHL* alleles in their tumors. [M. Marino, National Cancer Institute]

the two smaller subunits, B and C, are positive regulators of Elongin A function. They also demonstrate that the Elongin subunit B associates with Elongin C, and the Elongin B/C complex then binds to Elongin A. As shown by Duan *et al.* (*3*), the wild-type form of VHL also binds to Elongin B/C. Elongin A does not associate with VHL, suggesting that binding of VHL or Elongin A to the two regulatory subunits is mutually exclusive. Binding of VHL to the Elongin subunits B and C is mediated by a region that is homologous between VHL and Elongin A, and the binding of VHL to the B/C complex disrupts Elongin function. Forms of VHL that are mutated in this region of homology are found in patients with von Hippel–Lindau syndrome, and these mutants do not form a complex with Elongin B/C. This suggests that the interference of VHL with Elongin's transcriptional elongation activity is the basis of its tumor-suppressor (*5*) function. Importantly, Kibel *et al.* (*4*) demonstrate the VHL–Elongin B/C interaction that occurs in vivo.

These observations raise some interesting and important questions for research in basic molecular biology and molecular medicine. (i) Which gene or subset of genes is targeted by VHL? Clearly, because cells expressing mutated VHL are viable, VHL is not a global regulator of transcriptional elongation. However, several genes implicated in pathogenesis of malignancies, such as c-*myc* or others (for example, N-*myc*, L-*myc*, and c-*fos*) are regulated at the level of elongation (*2*) and thus are prime candidates for targets of VHL. (ii) Does VHL target other biochemical pathways? Some of the mutated forms of VHL found in renal carcinomas can bind to Elongin B/C, but the effect of these mutants on transcriptional elongation is unknown. Interestingly, VHL can bind to several other, as yet unidentified, cellular proteins, suggesting that VHL may have additional roles in cellular metabolism (*14*). (iii) How does Elongin function? Elongin activity has been demonstrated in deoxycytidine-tailed template assays in which naked DNA templates are transcribed by purified RNA polymerase II in the absence of transcription initiation factors (*13*). This observation suggests that Elongin interacts directly with nascent RNA, the RNA polymerase II complex, or both. The importance of nascent RNA in transcription elongation and termination has been demonstrated in prokaryotes (*15*), and the marked sequence similarity of Elongin C and the RNA-binding region of the *Escherichia coli* termination factor Rho suggests that binding of nascent RNA may be at least one aspect of Elongin function. In addition, pausing and termination in prokaryotes depend on discontinuous, inchworm-like movement of RNA polymerase, which results in dramatic compression of polymerase (*16*). Thus, Elongin may directly associate with RNA polymerase to inhibit "inchworming" or to catalyze conformational changes in RNA polymerase that permit rapid escape out of the pause site. In the presence of Elongin and absence of the wild-type VHL, the escape of RNA polymerase out of pause sites occurs at a much higher rate. The net result is accelerated movement of RNA polymerase through the gene.

The new results reported in this issue are not just a coup for cancer research. The identification and cloning of the *VHL* gene and its target Elongin will help to elucidate transcriptional elongation, a key regulatory mechanism. Even though differences in RNA polymerase elongation rates have been observed in in vitro transcription systems, the lack of appropriate assay systems has limited a more critical examination of the mechanism in vivo. We will now be able to move forward without pause in understanding how transcriptional elongation regulates gene expression, cell growth, and neoplasia.

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