PERSPECTIVES: TRANSCRIPTION

New Insights into an Old Modification

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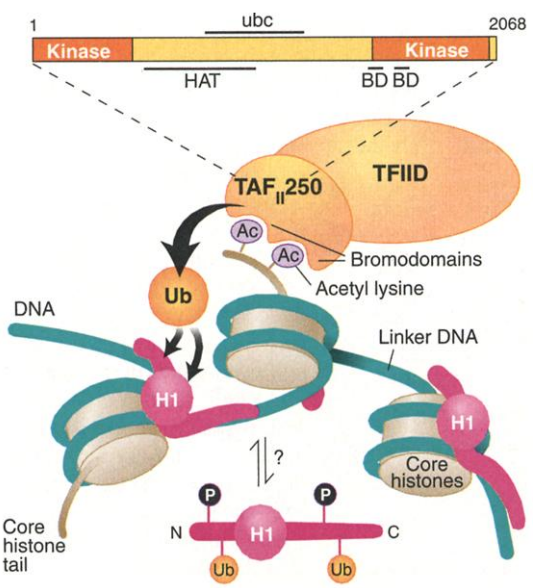
Genomic DNA within the nucleus of every cell contains the genes that specify the entire set of proteins involved in all cellular processes. The timing and rate of expression of groups of genes in different tissues is essential for normal growth and development; inappropriate gene expression can lead to cancer and other diseases. Transcription is the process whereby the DNA sequence of a gene is copied into mRNA, the template for protein synthesis. This process is initiated by the assembly of a large transcriptional complex at a promoter, a segment of DNA near the beginning of a gene that usually includes the characteristic DNA sequences known as the TATA box. In addition to the enzyme that makes the mRNA template (RNA polymerase II), the transcription complexes for all protein-coding genes contain a group of general transcription factors: TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH. Writing on page 2357 of this issue, Pham and Sauer (*1*) propose that a subunit of TFIID called $TAF_{II}250$ regulates gene transcription by enzymatically modifying the H1 histone protein in chromatin, thus altering the accessibility of chromatin to the transcriptional complex.

The general transcription factor TFIID is a multimeric complex that contains the TATA box binding protein (TBP) and a number of TBP-associated factors (TAF_{II}s). Binding of TFIID to a gene's promoter initiates the assembly of the other general transcription factors and RNA polymerase II into the transcription complex. This is a key step in gene transcription because TFIID is the only general transcription factor that is capable of directly binding to promoter DNA (*2*). Promoter recognition by TFIID can be likened to a game of "hide and seek" in the genome because promoters are dispersed throughout the vast expanse of mostly noncoding DNA and can be further obscured by the high degree of DNA folding, which results from the wrapping of DNA around the histone proteins in chromatin (see the figure).

Histones and other chromatin-associated proteins are known to be modified by the enzymatic activities of some transcriptional regulatory proteins. It is thought that these modifications influence promoter accessibility through direct effects on chromatin folding or by modulating the recruitment and activity of regulatory factors at chromatin loci. In their study, Pham and Sauer (*1*) demonstrate that $TAF_{II}250$ tags histone H1 with a ubiquitin marker and that this ubiquitination step is essential for expression of genes in the correct order during development of the fruit fly *Drosophila*. They propose a previously unsuspected role for H1 ubiquitination in transcriptional regulation through the remodeling of chromatin structure, which alters the accessibility of gene promoters to the transcription complex.

The core histones—H2A, H2B, H3, and H4—together with DNA form nucleosomes, the basic structural unit of chromatin. Two of each type of core histone form an octamer around which the DNA is wrapped. The amino-terminal "tails" of the eight core histones projecting from each nucleosome contain amino acids that can be targeted for covalent modification by a growing list of transcriptional regulators (see the figure). These modifications appear to modulate interactions between histone tails and other chromatin components. Such modifications promote unfolding of nucleosomal fibers and may also unmask binding sites for TFIID or for gene-specific transcription factors that are obscured by the binding of unmodified histone tails to DNA. For example, acetylation of lysine residues in core histones by histone acetyltransferases (HATs) neutralizes their positive charge, promoting decondensation of nucleosomal fibers and enhancing the accessibility of chromatin to the transcriptional machinery. $TAF_{II}250$ itself has HAT activity, although the natural targets of this activity have not yet been established. Conversely, removal of acetyl groups by histone deacetylases (HDACs) results in condensation of chromatin and decreased accessibility of chromosomal loci to transcription complexes (*3*). Furthermore, acetylated lysines are bound specifically by the bromodomain, a structural motif present in many transcriptional regulators including HATs such as $TAF_{II}250$ (*4*) and p300/CBP associated factor (*5*). This hints at the existence of a histone modification "code" that directs when and where specific regulatory proteins can bind to chromatin (*6*).



Reaching the core of transcription. $TAF_{II}250$, a subunit of the general transcription factor TFIID, modifies chromatin-associated proteins in several ways. *Drosophila* $TAF_{II}250$ has two bromodomains (BD) and domains possessing protein kinase (kinase), histone acetyltransferase (HAT), and ubiquitin-conjugating (ubc) activities. $TAF_{II}250$ binds to the acetylated (Ac) tails of core histones through its bromodomains. This interaction may enable $TAF_{II}250$ to ubiquitinate the H1 linker histone, resulting in an alteration in chromatin structure and the facilitation of transcription. Modification of histones or other chromatin-associated proteins through the kinase and acetyltransferase (HAT) activities of $TAF_{II}250$ may also be important for transcriptional regulation.

The accessibility of gene promoters is also regulated by higher-order chromatin folding mediated, in part, by linker histones of the H1 family. The binding of H1 histones to DNA extending between adjacent nucleosomes is thought to facilitate folding of the DNA and chromatin condensation (see the figure). In transcriptionally active chromatin, it has been established that either H1 is depleted or its binding to DNA is altered, but how this is achieved is unclear. H1 is phosphorylated at multiple sites during the cell cycle; one possible way in which chromatin condensation could be reduced is by weakening of H1:DNA interactions through phosphorylation of serine and threonine residues in H1 tails. It seems that phosphorylation acts primarily by altering the overall charge within small domains of H1 (*7*). H1 phosphorylation affects transcription in a gene-specific manner (*8*), and increased amounts of phosphorylated H1 have been

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found in cells transformed by certain oncogenes (*9*, *10*).

The gene encoding $TAF_{II}250$ was identified long before any clear evidence emerged for its role in transcriptional regulation. A wild-type copy of the gene—*CCG1* (cell cycle gene 1)—from normal cells rescued the G_1 phase arrest of cells bearing a *CCG1* point mutation (*11*). It turns out that the protein encoded by *CCG1*, $TAF_{II}250$, is a critical subunit of TFIID and integrates interactions between transcription factors and various TAF_{II}s, enabling their communication with TBP, the DNA binding component of TFIID (*2*). The discovery that $TAF_{II}250$ possesses both acetyltransferase and kinase activities (*12*, *13*) suggests a catalytic as well as a structural role for this subunit in gene expression. Intriguingly, cells with mutations that affect the HAT activity of $TAF_{II}250$ become arrested in late G_1 of the cell cycle, suggesting that $TAF_{II}250$ regulates the expression of genes involved in cell proliferation (*14*).

The Pham and Sauer results are unexpected because they point to an even greater diversity in the enzymatic capabilities of $TAF_{II}250$ than previously surmised. Although ubiquitination is most frequently associated with degradation of proteins through the proteasome pathway (*15*), ubiquitinated H2A and H2B are known to be enriched in transcriptionally active chromatin (*16*, *17*). Pham and Sauer show that the ubiquitin-conjugating activity of $TAF_{II}250$ is associated with H1 ubiquitination in *Drosophila* embryos. Their data suggest that ubiquitination alone, or in combination with the modification of other chromatin-associated proteins, may alleviate repression of transcription by H1. This raises several intriguing questions: To what extent do the HAT and kinase activities of $TAF_{II}250$ influence H1 ubiquitination and vice versa? Do distinct combinations of different histone modifications form a code that influences which sets of genes are transcribed (*6*, *18*)? Identification of the H1 sites that are ubiquitinated by $TAF_{II}250$ may reveal synergy between H1 phosphorylation and H1 ubiquitination.

Other important issues raised by this work include determining the effect of H1 ubiquitination on chromatin structure, whether ubiquitination influences the proteolytic turnover of H1 in a locus-specific manner, and whether H1 is ubiquitinated in other organisms. Chromatin remodeling varies with the different stages of the cell cycle (*19*). Thus, the initial description of $TAF_{II}250$ as a cell cycle regulator begs for a careful analysis of the cell cycle dependency of H1 ubiquitination and of the HAT and kinase activities of this important TFIID subunit. Even though more work is required to elucidate the importance of HI ubiquitination, this addition to the list of $TAF_{II}250$'s chromatin modifying activities provides further evidence for the key part played by this molecule in gene transcription.

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PERSPECTIVES: ATMOSPHERIC CHEMISTRY

The NO_2 Flux Conundrum

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Striking progress has recently been made in understanding the central role of nitrogen oxide radicals, NO_x, in atmospheric processes. NO_x is implicated in the formation of acid rain, tropospheric ozone (the principal toxic component of smog and a greenhouse gas), and the hydroxyl radical (the main atmospheric oxidant responsible for the destruction of many pollutants). Atmospheric models have had some success at reproducing regional and continental acid deposition patterns, ozone profiles, and hydroxyl radical concentrations on the basis of estimated NO_x emissions (*1*–*3*). However, atmospheric and biological studies have yielded seriously incompatible results regarding the role of vegetation as a sink or source of NO_x. This is an important problem because we must understand NO_x emission processes to be able to predict future environmental impacts (*4*–*6*).

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The major known sources of NO_x are fossil fuel combustion, biomass burning, microbial activity in soils, and lightning. Globally, these sources produce a total of 30 to 50 teragrams (Tg) of nitrogen $year^{-1}$ of which microbes in soils contribute 5 to 10 Tg $year^{-1}$. The vast majority of NO_x is released as nitric oxide, NO, which converts to nitrogen dioxide, NO_2, within minutes by reaction with ozone and peroxy radicals. NO_2 is recycled to NO by photolysis. This cycle is at the heart of tropospheric ozone formation. Typical NO/NO_2 concentration ratios in surface air are 0.2 to 0.5 in the daytime and zero at night when no NO_2 photolysis takes place. Over time scales of hours to days, NO_x is converted to nitric acid and nitrates, which are removed by rain and dry deposition and contribute to acidification and excess nutrients in sensitive ecosystems.

NO_x is also removed directly from the air through uptake of NO_2 by foliage. This process extracts NO_x from the atmosphere and also removes soil-derived NO_x from the air before it can be exported to the atmosphere. The efficiency of the latter process is crucial for determining the NO_x concentration above landscapes dominated by biological activity. A quantitative analysis of this effect was made by Jacob and co-workers (*7*, *8*) using data from an Amazonian forest site during the wet season. The authors modeled observed NO_x concentrations in the canopy air with a one-dimensional atmospheric transport and chemistry model constrained by measured NO soil emission fluxes and estimated that only 25% of the NO_x emitted by soils is ventilated to the atmosphere. Globally, the fraction of soil-derived NO_x ventilated out of canopies has been estimated at 50 to 80% (*9*, *10*) by extrapolating Jacob and co-workers' results to canopies of different leaf area indices.

The kinetics of NO_2 uptake by plants have been studied by biologists interested in NO_2 exchange mechanisms and the impact of NO_2 on plant function. In these bottom-up studies, leaf-level exchange of NO_2 is measured across a range of concentrations, and a "compensation point" is calculated assuming first-order uptake kinetics. At ambient concentrations below the compensation point, the plant canopy is a net source of NO_2 to the atmosphere, whereas at concentrations above this point, it acts as a net sink. Most studies of leaf-level NO_2 exchange have shown compensation points between 1 and 3 parts per billion by volume (ppbv) (*11*–*15*). These results contradict those of Jacob and co-workers (*7*, *8*), who found that at NO_2 concentrations as low as 0.2 to 0.4 ppbv in the canopy air, rapid net uptake of NO_2 by the leaves was

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