Unlocking the Gates to Gene Expression

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Recent research on long-chain complexes between polyradicals or carbenes and paramagnetic transition metal ions has led to superhigh-spin molecules with antiferromagnetic (Mn^{2+}) or ferromagnetic (Cu^{2+}) behavior at cryogenic temperatures (*19*). In these complexes, the organic moieties are carriers of the free spins as well as ligands of the paramagnetic metal ions.

The availability of stable diradicals at room temperature (*6*) will lead to attempts to generate stable polyradicals with ferromagnetic or antiferromagnetic properties. Work in other laboratories suggests that it may be possible to exploit substituent effects to induce singlet or triplet ground states of localized diradicals (*5*, *15*). It may be possible to link such localized diradicals in polymer or dendrimer chains. The scientific community will await further reports on the chemical and physical behavior of the new diradicals with keen interest.

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PERSPECTIVES: TRANSCRIPTION

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How activators regulate gene transcription has been hotly debated for more than a decade (*1*). Initial studies suggested that activator proteins bound to the promoter of the target gene might activate transcription by making contact with one or two key proteins within the core transcription machinery. However, it is now clear that activators must orchestrate the recruitment of numerous proteins including chromatin-remodeling enzymes before gene transcription can proceed. How is the recruitment of chromatin-remodeling enzymes coordinated with assembly of the core transcription machinery, and which transcription steps are regulated by these enzymes? Findings reported by Soutoglou and Talianidis (*2*) on page 1901 of this issue, together with other work, reveal that chromatin-remodeling enzymes can regulate nearly every step of the pathway leading to gene transcription.

In eukaryotes, genomic DNA is organized into chromatin. The basic subunit of chromatin, the nucleosome, is composed of ~147 base pairs of DNA wrapped around a complex of eight histone proteins. The most simple form of chromatin contains genomic DNA packaged into nucleosomes to form long strands that resemble beads on a string. The organization of chromatin poses a barrier to transcription because it prevents the transcription machinery from interacting directly with promoter DNA sequences. Given that chromatin in vivo is further folded into compact fibers (30 to 400 nm thick), how can the transcription machinery possibly access the genes hidden within the nucleosomal milieu? The solution lies in chromatin-remodeling enzymes that alter the folding, fluidity, and basic structure of chromatin. There are two classes of chromatin-remodeling enzymes: those that covalently modify nucleosomal histone proteins through acetylation, phosphorylation, or methylation, and those that alter chromatin structure through hydrolysis of the energy-rich molecule adenosine triphosphate (ATP) (*3*). Certain histone-modifying enzymes, such as the histone acetyltransferases (HATs) Gcn5p and P/CAF, and some ATP-dependent remodeling enzymes, such as SWI/SNF, directly interact with gene-specific activators to ensure that chromatin remodeling is targeted to the correct gene, in the proper cell, and at the right time.

In the early days of transcription research when chromatin was largely ignored, an activator was presumed to enhance transcription by promoting recruitment of proteins to the gene promoter and directing their assembly into a preinitiation complex (PIC), composed of RNA polymerase II and other general transcription factors. A more modern view is that activators must first recruit chromatin-remodeling enzymes in order to create a chromatin environment permissive for PIC assembly. This view, still too simplistic, may be valid only for in vitro systems, artificial reporter genes, and a small subset of endogenous genes. As illustrated in the following examples, it is now clear that other factors, such as the chromatin structure of the gene promoter and the phase of the cell cycle, also govern how chromatin-remodeling enzymes collaborate with each other to control steps before, during, or after PIC assembly.

Although the yeast *HO* gene is transcribed during G_1 phase of the cell cycle, the Swi5p activator recruits the SWI/SNF chromatin-remodeling complex to the *HO* upstream regulatory region during late mitosis of the previous cell cycle (*4*). Surprisingly, SWI/SNF activity is absolutely required for recruitment of the HAT complex Gcn5p, which also occurs during the previous mitosis (*4, 5*). SWI/SNF action and Gcn5p-dependent histone acetylation facilitate the binding of a second gene-specific activator, SBF, to chromatin. Finally, RNA polymerase II and other general transcription factors are recruited, resulting in completion of PIC assembly and the initiation of *HO* gene transcription (see the figure, A). Thus, at the *HO* promoter, SWI/SNF action controls HAT recruitment, and subsequent chromatin remodeling governs the binding of an activator, a very early step in transcriptional activation. Interestingly, the interdependence of SWI/SNF and the Gcn5p HAT may be a general property of genes expressed at the end of mitosis (*6*). Condensation of the chromosomes during mitosis may confer an obligatory functional relationship on these two enzymes.

Activation of the human interferon-β (IFN-β) gene promoter involves a very different order of events (*7*). In this case, viral infection of human cells generates a signal that induces the binding of a group of activators to a nucleosome-free region of DNA upstream of the *IFN-β* gene. This DNA-activator complex, the enhanceosome, first promotes the rapid recruitment of the Gcn5p HAT, which acetylates nucleosomes encompassing the TATA box in

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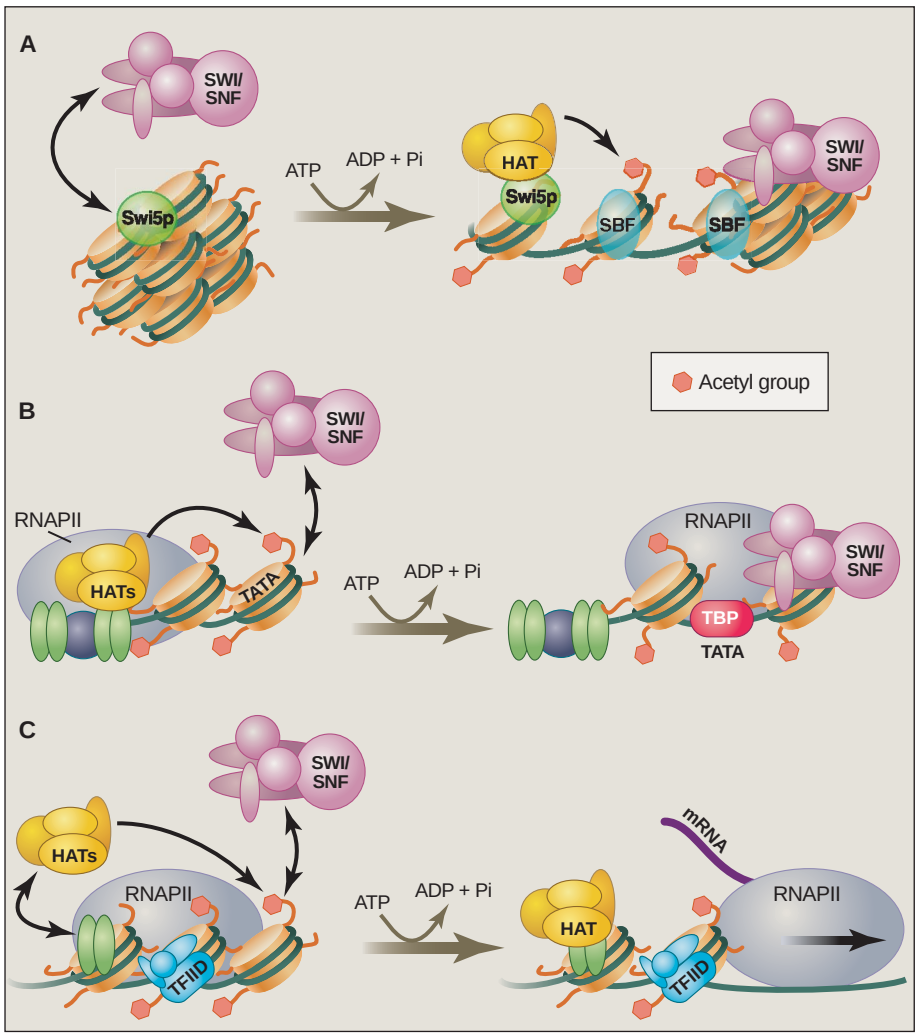

The complexities of gene transcription. Chromatin-remodeling enzymes facilitate multiple steps during the activation of gene expression. (**A**) At the yeast *HO* gene promoter, the Swi5p activator recruits the SWI/SNF and Gcn5p HAT chromatin-remodeling complexes very early in gene activation, before PIC assembly begins. SWI/SNF controls recruitment of the HAT complex, possibly by destabilizing chromatin condensation. SWI/SNF and the HAT complex then cooperate to facilitate binding of a second activator, SBF. (**B**) At the human *IFN-β* gene promoter, upstream activators (green and purple) recruit multiple HAT proteins during assembly of the PIC, which contains RNA polymerase II (RNAPII). Subsequent histone acetylation promotes the recruitment of the SWI/SNF complex, which disrupts the structure of a promoter-bound nucleosome. This facilitates TBP binding to the TATA element, which completes PIC assembly. (**C**) Multiple HAT complexes (CBP and P/CAF) and the SWI/SNF complex are recruited to the human α_1-*AT* gene promoter after PIC assembly, stimulating subsequent expression of the α_1-*AT* gene.

the gene's promoter. Nucleosome acetylation directs the stable recruitment of a SWI/SNF complex, probably by stabilizing weak interactions between this complex and the enhanceosome. ATP-dependent remodeling by SWI/SNF alters the structure of the promoter-bound nucleosome, enhancing binding of the TATA-box binding protein (TBP) to the TATA element, thereby resulting in the completion of PIC assembly (see the figure, B). So, in this case, a HAT chromatin-remodeling enzyme facilitates recruitment of SWI/SNF, and chromatin-remodeling activity drives PIC assembly on the nucleosomal *IFN-β* gene promoter.

Investigating expression of the α_1-antitrypsin (α_1-*AT*) gene during gut cell differentiation, Soutoglou and Talianidis (*2*) have discovered a third way in which chromatin-remodeling enzymes regulate gene transcription. Unlike the *IFN-β* gene promoter, the entire region upstream of the α_1-*AT* gene, including binding sites for TBP and activators, contains nucleosomes. Furthermore, one activator protein, HNF-1, and two general transcription factors, TBP and TFIIB, are already bound to the nucleosomal promoter region even in the absence of differentiation signals or recruitment of chromatin-remodeling enzymes. When gut cell differentiation is induced, RNA polymerase II and other general transcription factors are rapidly recruited to the PIC in the absence of chromatin-remodeling enzymes. Thus, contrary to the other two models, it appears that an entire PIC can be assembled onto a nucleosomal promoter without prior chromatin remodeling! Why the PIC forms on the nucleosomal α_1-*AT* promoter in the absence of remodeling but not at the promoters of other genes such as *IFN-β* remains a mystery.

Does chromatin even participate in the regulation of α_1-*AT* gene transcription? Of course it does! A SWI/SNF-like enzyme and two HATs (CBP and P/CAF) are recruited to the promoter region after PIC assembly, possibly by another activator, HNF-4. Promoter-associated nucleosomes are disrupted by histone acetylation and SWI/SNF action, resulting in α_1-*AT* transcription. The SWI/SNF-associated chromatin reconfiguration occurs after phosphorylation of the carboxyl-terminal domain of RNA polymerase II in the PIC, strongly suggesting that SWI/SNF is involved either late in transcription initiation or during elongation of the mRNA. This finding parallels earlier studies suggesting that SWI/SNF releases paused mRNA elongation complexes during transcription of the human *HSP70* gene in vitro (*8*).

Thus, chromatin-remodeling enzymes can regulate key steps in transcription before, during, and after assembly of the PIC at the target gene promoter. But what determines whether chromatin remodeling is required at a specific gene? In yeast, the global condensation of chromatin during mitosis confers a general requirement for chromatin remodeling during the very early steps of transcriptional activation. Less clear, however, are the factors determining whether chromatin remodeling is required for PIC formation or for subsequent steps in transcription. Both the *IFN-β* and α_1-*AT* gene promoters are encompassed by nucleosomes, but chromatin remodeling prior to PIC assembly is only required at the *IFN-β* gene promoter. So it is not simply the presence of nucleosomes surrounding promoter sequences that dictates whether chromatin remodeling is required. Clearly, we still have much to learn about how activators orchestrate events leading to the transcriptional activation of endogenous genes and the part played by chromatin remodeling in this process.

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