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# Fundamentally Different Logic of Gene Regulation in Eukaryotes and Prokaryotes

Minireview

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The basic principles of gene regulation were established by the mid-1960s, primarily by the pioneering work of Francois Jacob and Jacque Monod. The fundamental units of gene regulation are the three types of specific DNA sequences that determine the level of expression under particular physiological conditions. Promoters, originally defined as elements that determine the maximal potential level of gene expression, are recognized by RNA polymerase and contain all the information necessary for accurate transcriptional initiation. Operator sequences are recognized by repressor proteins which inhibit transcription that would otherwise occur from the promoters. Lastly, as initially recognized by Ellis Englesberg, positive control elements are recognized by activator proteins that stimulate transcription from the promoter. The function of activators and repressors can be modulated by specific physiological conditions, thereby permitting regulated expression of the cognate genes.

The advent of recombinant DNA technology 25 years ago made it possible to ask whether prokaryotic paradigms of regulatory logic and molecular mechanism could account for the vastly increased complexity of eukaryotic organisms. Indeed, it is now clear that a remarkable number of basic principles are universal. Nevertheless, I will argue that the logic of gene regulation in prokaryotes and eukaryotes is fundamentally different. This difference in logic reflects important differences in transcriptional regulatory mechanisms, the most important of which arises from the fact that eukaryotic DNA is packaged into chromatin templates. These fundamental differences are essential for eukaryotic organisms to express genes in the incredibly diverse patterns that are necessary for biological complexity.

### *Prokaryotic Organisms: The Ground State Is Nonrestrictive*

The concept of a transcriptional ground state is useful in understanding the logic of gene regulation at the level of the intact organism. In this review, I will define the transcriptional ground state as the inherent activity of promoters (and hence core transcription machineries) in vivo in the absence of specific regulatory sequences (and hence activators and repressors). This concept is independent of the strength/quality of individual promoters, which will vary considerably as a function of the DNA sequence.

Prokaryotic RNA polymerases recognize promoters via specific sequences immediately upstream of the initiation site. In vitro, transcription is initiated efficiently on purified DNA templates, with the rate and level of transcription being determined simply by the quality of the promoter sequences (which is related, but not equivalent to, DNA binding affinity). In vivo, an isolated promoter region is sufficient to initiate transcription at a rate comparable to that achieved in vitro. Moreover, the strongest promoters initiate transcription at the maximal rate, which is ultimately limited by the rate at which RNA polymerase elongates and clears the promoter, thereby permitting another round of initiation. In other words, there is no inherent restriction on the ability of prokaryotic RNA polymerase to gain access to the DNA template and initiate transcription in vivo. Thus, for prokaryotic organisms, the ground state for transcription is nonrestrictive (Figure 1).

The nonrestrictive ground state has important implications for the mechanisms by which prokaryotic repressors and activators affect transcription as well as for

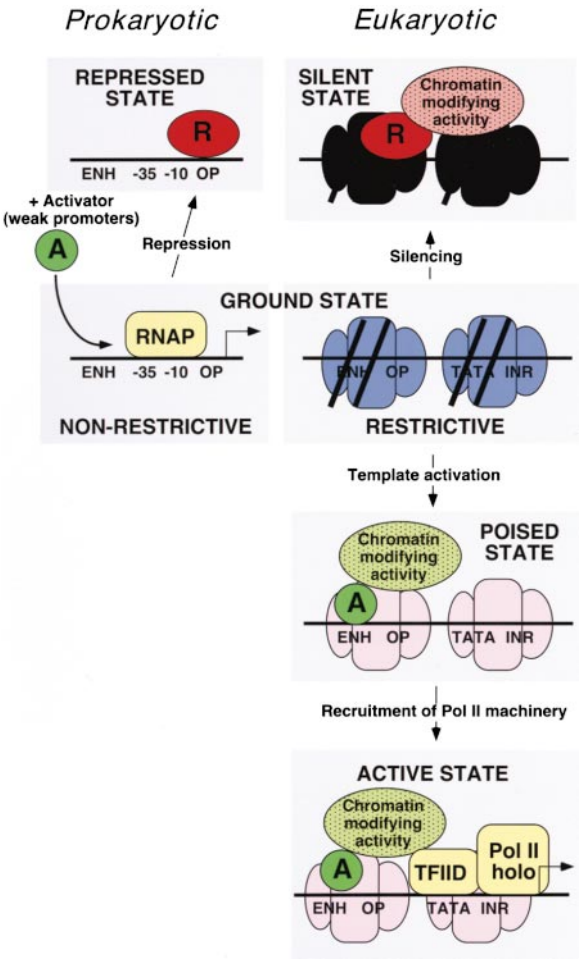


Figure 1. Transcriptional States in Prokaryotes and Eukaryotes

Activators (A) and repressors (R) interact respectively with enhancer (ENH) or operator (OP) sequences and affect transcription by prokaryotic RNA polymerase (RNAP) or the eukaryotic Pol II machinery (TFIID + Pol II holoenzyme). In eukaryotes, recruitment of chromatin modifying activities by activators or repressors leads to altered chromatin structure (depicted by color or DNA within nucleosomes). See text for details.

the regulatory logic at the genomic level. Repressors function by blocking the activity of RNA polymerase, and this can occur by simple occlusion of RNA polymerase binding to the promoter or by the generation of repressosome structures in which interactions between repressor molecules bound at distinct sites cause DNA loops (Hochschild and Dove, 1998; Geanacopoulos et al., 1999). Although repressors are utilized at a subset of promoters, they are required to keep gene activity at a low level, except in the cases where the promoter itself is very weak.

For genes regulated by activators, the promoters must be inherently weak (or specifically repressed) because it is impossible to increase the activity of a strong promoter beyond the maximal initiation rate. Activators stimulate transcription by directly interacting with RNA polymerase, and at least 3 of the 4 subunits ($\alpha$, $\sigma$, $\beta'$) are physiologically relevant targets (Hochschild and Dove, 1998). These activator interactions can increase association of RNA polymerase with the promoter via cooperative DNA binding, or they can stimulate the activity of RNA polymerase that is already bound to the promoter. Importantly, activators are not universally required for transcription in vivo, and hence are utilized only at a subset of prokaryotic promoters.

In prokaryotes, the genomic DNA is associated with histone-like proteins in a structure termed the nucleoid. This nucleoid structure is unlikely to play a general inhibitory role in transcription because simple promoters are easily accessible and fully functional in vivo. However, histone-like proteins are required for repression of certain promoters by virtue of their presence in repressosome complexes that inhibit access of RNA polymerase to the promoter (Schnetz and Wang, 1996; Geanacopoulos et al., 1999). In these situations, the histone-like proteins do not have a general inhibitory function, but instead play a specific architectural role (Werner and Burley, 1997) for repressosome formation.

### *Eukaryotic Organisms: The Ground State Is Restrictive*

In eukaryotes, the basic transcription machinery is a ribosome-sized entity with two basic components, TFIID and the Pol II holoenzyme. TFIID binds specifically to TATA promoter elements in a manner loosely analogous to prokaryotic $\sigma$ factors. The Pol II holoenzyme, loosely defined, consists of the core RNA polymerase II, general transcription factors, and other associated proteins; it has limited DNA sequence specificity, but interacts specifically with promoters via its interactions with TFIID. In vitro, the basic Pol II machinery is sufficient for efficient and accurate initiation from a core promoter containing TATA and initiator elements. However, in striking contrast to the situation in prokaryotes, a strong core promoter is essentially inactive in eukaryotic cells. Thus, the ground state for transcriptional activity in eukaryotic cells is restrictive (Figure 1).

The fact that core promoters are virtually inactive means that transcription of essentially all eukaryotic genes requires activators. Conversely, repressors are not required to keep the level of gene activity at a low level, although they are utilized at certain promoters. Efficient transcription generally requires the synergistic action of multiple activators bound at distinct sites upstream (or downstream) of the promoter. Activator-binding sites are often clustered into enhancers that function as autonomous regulatory units, and in some cases multiple activators interact to form a highly structured protein–DNA complex termed the enhanceosome (Carey, 1998). Thus, transcriptional activation in eukaryotes is inherently combinatorial; each of the large number of possible combinations is biologically distinct, and an individual core promoter can be regulated with remarkable diversity and precision.

### *Chromatin Maintains the Transcriptionally Restrictive Ground State*

Although chromatin is often viewed as a general inhibitor of protein access to DNA, the degree to which nucleosomes inhibit DNA-binding proteins from interacting with their cognate sites is highly variable (Workman and Kingston, 1998). Of particular significance, nucleosomes virtually prevent the binding of TBP to the TATA element in vitro, and TBP does not associate with the vast majority of yeast core promoters in vivo in the absence of a functional activator (Kuras and Struhl, 1999; Li et al., 1999). The inability of TBP to bind nucleosomal DNA means that the entire basic transcription machinery is excluded from simple chromatin templates. On the other hand, nucleosomes have only a modest inhibitory effect on the ability of a variety of activator proteins to bind their target sites. Thus, chromatin maintains the restrictive ground state by blocking the association of the basic Pol II machinery with the core promoter, while permitting many activators to bind their target sites.

There are two classes of mechanisms by which eukaryotic activators could enhance the association of the Pol II machinery with promoters. First, activators could directly interact with components of the Pol II machinery. Such direct recruitment is analogous to the activation mechanism in prokaryotes, although there are many more potential targets in the Pol II machinery (Ptashne and Gann, 1997). Second, in a mechanism specific to eukaryotes, activators could indirectly increase recruitment of the transcription machinery by altering chromatin structure.

### *Gene Regulation by Targeted Recruitment of Chromatin Modifying Activities*

A simple, yet powerful, model for linking classical transcriptional regulatory mechanisms with chromatin structure is that activators and repressors can recruit chromatin modifying activities to promoters. The first example of such a mechanism involves repression by the Sin3-Rpd3 histone deacetylase complex. This complex is recruited to specific promoters by DNA-binding repressors, whereupon it generates a highly localized domain of histone deacetylation that spans 1–2 nucleosomes (Kadosh and Struhl, 1998; Rundlett et al., 1998). Thus, the DNA-binding repressor does not directly inhibit the transcription machinery, but rather creates a localized domain of repressive chromatin structure that indirectly (by unknown mechanisms) reduces Pol II transcription.

It is now clear that eukaryotic activators also function, at least in part, by recruiting chromatin modifying activities to promoters. Gcn5 histone acetylase specifically acetylates histones in the vicinity of an activated promoter (Kuo et al., 1998), and activators can interact directly with histone acetylase or nucleosome remodeling complexes and stimulate transcription on chromatin, but not purified DNA templates in vitro (Utley et al., 1998; Kingston, 1999). Most convincingly, as discussed below, the Swi5 activator is required for recruitment of both the Swi/Snf nucleosome remodeling complex and the

SAGA histone acetylase complex to the *HO* promoter (Cosma et al., 1999), and Swi5-dependent histone acetylation of the *HO* promoter occurs prior to, and independent of, transcription (Krebs et al., 1999). It is presumed that activator-dependent modification of chromatin indirectly increases recruitment of the Pol II machinery to promoters.

### *Ordered Recruitment of Chromatin Modifying Activities*

The activation of developmentally regulated genes often occurs after a series of changes in chromatin structure, although the underlying mechanisms are poorly understood. In this regard, studies on the cell cycle– and developmentally regulated yeast *HO* gene have been illuminating and provocative. *HO* expression is restricted to a short time in late G1, and it occurs in mother cells but not in daughter cells. *HO* transcription requires both the Swi/Snf and SAGA complexes, as well as at least two activators, Swi5 and SBF. Interestingly, high levels of Swi5 are present only for a short time as cells are dividing, which is considerably before late G1 when *HO* is transcribed.

The association of these activators and chromatin modifying activities with the *HO* promoter were directly measured by chromatin immunoprecipitation (Cosma et al., 1999). The first step of *HO* activation occurs when Swi5 binds to sites within the URS1 element located at −1300 with respect to the initiation site. Swi5 association occurs in the absence of Swi/Snf and SAGA, presumably because it is not severely inhibited by chromatin templates. Swi5 binding is required for the association of the Swi/Snf complex with URS1, which occurs almost immediately thereafter; five minutes later, Swi/Snf associates with URS2, an element located at −700. This Swi5-dependent recruitment of Swi/Snf is required for the near concurrent association of SAGA with URS2; conversely, Swi/Snf association occurs in the absence of SAGA. Finally, SBF binds to URS2, but this occurs only after both chromatin modifying activities have been recruited to the *HO* promoter. This linear pathway of ordered recruitment—Swi5, Swi/Snf, SAGA, SBF—occurs only in mother cells. In daughter cells, the Ash1 repressor associates with the *HO* promoter shortly after Swi5 binding, whereupon it blocks the recruitment of Swi/Snf and hence all subsequent events.

A complementary analysis of histone acetylation of the *HO* promoter reveals that a domain of histone H3 and H4 acetylation is established in mid-G1, which is prior to and independent of *HO* transcription (Krebs et al., 1999). Unlike histone acetylation at the *his3* promoter, which occurs over a range of 1–2 nucleosomes, the domain of acetylation at the *HO* promoter is much larger, occurring over a 1 kb region. Strikingly, histone acetylation at URS2 occurs prior to that observed in the region around the TATA element, suggesting that SAGA is initially recruited to URS2 whereupon it travels downstream.

### *Novel Mechanisms of Transcriptional Activation in Eukaryotes: Epigenetic Memory, Poised States, and Creation of Extended Domains of Active Chromatin*

Several new principles of eukaryotic gene regulation have been established from these studies on the *HO* promoter (Figure 2).

(1) Binding of an activator (e.g., Swi5) to its target site can be required for transcription, even though the activator is not associated with the promoter when transcription actually occurs. In such a case, the role of the activator is solely to recruit a chromatin modifying activity.

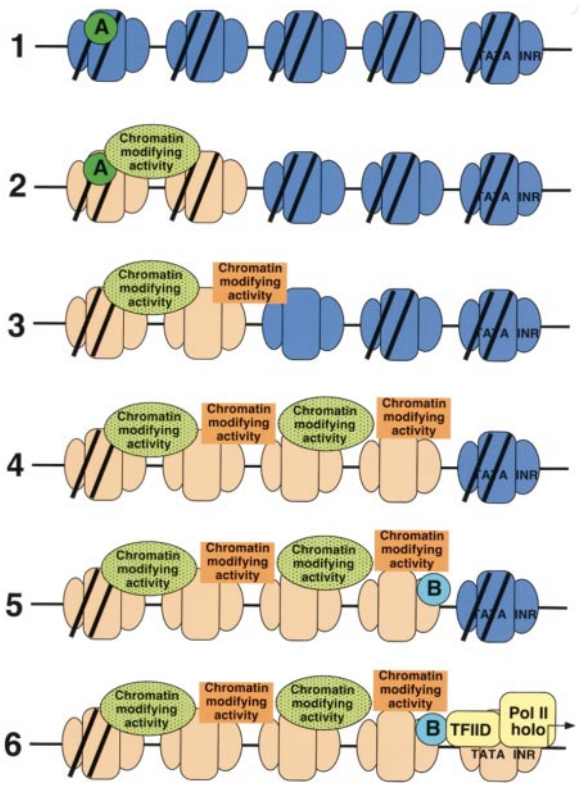


Figure 2. Hypothetical Intermediate States of Eukaryotic Transcription for a Given Promoter

Activator A binds to unmodified nucleosomal templates, leading to successive recruitment of chromatin modifying activities and the creation of an extended domain of active chromatin structure that permits binding of activator B and subsequent recruitment of the Pol II machinery. Binding of activator A is transient, but the recruited chromatin modifying activities remain stably associated for extended times. The existence, order, and stability of the indicated states can be developmentally regulated, but will vary according to the specific promoter.

(2) Swi/Snf (and presumably other chromatin modifying activities) can both be recruited to promoters by some activators (e.g., Swi5) and be required for the recruitment of other activators (e.g., SBF). This difference may reflect the ability of activators to bind nucleosomal templates; e.g., Swi5 binding might be relatively uninhibited by nucleosomes, whereas SBF binding might require chromatin remodeling.

(3) Chromatin modifying activities that are recruited to promoters by specific DNA-binding activators can remain associated with promoter for extended times even after the activator departs the scene. Hence, these chromatin modifying activities constitute a physical reminder or epigenetic memory of the initiating event.

(4) Recruitment of one chromatin modifying activity can be required for recruitment of a different chromatin modifying activity. More generally, chromatin modification at a promoter can occur in multiple steps that, in principle, can be temporally separated and independently regulated.

(5) The combined action of Swi5, Swi/Snf, and SAGA leads to a transcriptionally poised state; *HO* expression does not occur until SBF (and possibly other proteins) associates with promoter and stimulates the Pol II machinery. Thus, the generation of an active chromatin structure can be mechanistically and temporally distinct from the process of transcriptional activation per se.

(6) Chromatin modifying activities can progress from the upstream site of recruitment to more downstream regions including the core promoter, thereby leading to an expanding domain of active chromatin structure. Such long-range propagation of a wave of chromatin opening provides an alternative mechanism to the generally accepted, but not experimentally demonstrated, idea that activation at long distances from the promoter occurs by DNA looping resulting from protein–protein contacts between the activator and the Pol II machinery.

As will be discussed below, these new principles are of particular importance for gene regulation throughout development, and they have no obvious counterpart in prokaryotes. Aside from their biological significance, the detailed molecular mechanisms underlying these principles are of great interest, and they should be addressed experimentally in the near future.

### *Specialized Repression Mechanisms Are Required for Complete Silencing of Gene Expression*

In many biological situations, it is essential to silence completely the expression of particular genes. Although chromatin structure per se is responsible for maintaining a restrictive ground state, it is not sufficient to completely block normal or cryptic activators. As a consequence, eukaryotes have evolved specialized repression mechanisms in which a DNA-binding protein recruits protein complexes to particular regions of the genome, thereby creating an extended domain of modified chromatin structure. In the case of the yeast mating-type silencer, where the domain of "heterochromatin" is 5–10 kb, the DNA-binding protein Rap1 (together with associated corepressors) recruits a complex of Sir proteins which can self-associate into a polymer-like structure that spreads from the site of recruitment (Hecht et al., 1996). In worms, SDC-2 targets a specialized complex of dosage compensation proteins to hermaphrodite X chromosomes to reduce gene expression by half (Dawes et al., 1999). In these and other large-scale repression mechanisms, the ultimate repressor that recognizes selected sites or regions of the genome does not directly affect the transcription machinery, but rather the substrate for transcription.

### *Eukaryotic Regulatory Logic and Complex Gene Regulation*

The regulatory logic and underlying mechanisms of eukaryotic gene regulation are ideally suited for achieving extremely complex patterns of gene expression. Aside from the inherently combinatorial nature of gene activation, eukaryotic activators and repressors can function indirectly by recruiting chromatin modifying activities and hence creating domains of altered chromatin that vary in extent from individual nucleosomes to large genomic regions. Consequently, a eukaryotic promoter can exist in a variety of stable and intermediate states that are transcriptionally inactive (Figure 2); these states correspond to the chromatin modifying activities recruited by particular activators or repressors and hence can be regulated by cell cycle and developmental signals. The initiation, propagation, maintenance, and dissolution of such intermediate states can account for many important phenomena at the level of individual genes: transient initiating events playing a determining role in transcription at later stages of development; epigenetic memory through cell division cycles for maintenance of differentiated states; sequential opening of chromatin structure in complex genetic loci; generation of large-scale heterochromatic regions. It is hard to imagine such phenomena occurring in prokaryotes given that activators and repressors function rather directly on RNA polymerase in the context of a transcriptionally nonrestrictive ground state. Lastly, activator- and repressor-dependent recruitment of chromatin-modifying complexes may explain why regulation of other eukaryotic processes, such as DNA replication and site-specific recombination, is associated with transcriptional regulatory proteins, but not transcription per se.

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