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Commentary

Transcriptional regulation: Contending with complexity

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Regulation of gene expression is fundamental to biological systems, and much of this occurs at the level of transcription initiation. Consider the complexity of the transcriptional regulatory problem in the simple eukaryote *Saccharomyces cerevisiae*. Approximately 200 transcriptional activators are thought to be responsible for regulation of 6,200 genes (Fig. 1). At each gene, activators interact with one or more of ≈85 components in the initiation apparatus (1–4). Both activator and initiation apparatus must contend with nucleosomal DNA, and at least half a dozen chromatin modifying enzymes are involved (5–7). Various general and gene-specific negative regulators are also present (4, 8). The product of this collaboration among activators, the initiation apparatus, chromatin and its modifying enzymes, is an mRNA population whose individual levels range from <0.1 molecule/cell to >100 molecules/cell (9, 10). Cells efficiently remodel this population as they execute programs of growth control and differentiation and as they experience changes in their environment. Genome-wide monitoring of individual transcript levels during the mitotic cell cycle, nutrient deprivation, and sporulation shows intricate patterns of change within the population of mRNAs on a vast scale (11–13).

Precisely how transcriptional activators and the many components of the transcription initiation apparatus collaborate to regulate gene expression is a fundamental question in the field of eukaryotic gene expression. The mechanistic aspects of this question are best addressed in systems reconstituted *in vitro* with purified components, but the complexity of the transcription apparatus has made reconstitution of a physiologically relevant system particularly challenging. In this issue of the *Proceedings*, Myers *et al.* (14) take up the challenge with a transcription system from yeast that incorporates multiple activators and various forms of the RNA polymerase II holoenzyme.

The model shown in Fig. 2 summarizes some of the protein complexes thought to be involved in transcription initiation in yeast cells. Transcriptional activators bind to specific promoter sequences and recruit the initiation apparatus, presumably through physical interactions with components of the apparatus. The initiation apparatus includes RNA polymerase II, the Srb/Mediator complex, the Srb10 CDK complex, the Swi/Snf complex, and general transcription factors. Most of these components can be purified as a single complex from cells called an RNA polymerase II holoenzyme (refs. 15 and 16; reviewed in ref. 3), and transcription of most genes is initiated by the holoenzyme form of RNA polymerase II (10, 17). The subcomplexes of the holoenzyme can be separated from one another and purified to homogeneity, and this property has allowed investigators to study the contribution of individual subcomplexes in systems reconstituted with purified factors *in vitro*.

Several functions have been identified for (sub)complexes within the initiation apparatus. The Srb/mediator complex can reconstitute the response to activators in a system in which purified RNA polymerase II and general transcription factors cannot (18, 19). The Srb10 CDK complex functions as a repressor of a subset of genes during logarithmic growth in glucose; Srb10 is depleted from cells, and this set of genes is derepressed when cells experience nutrient deprivation (10, 20). The Swi/Snf complex plays a role in chromatin remodeling, thus altering nucleosomal DNA at promoters and possibly elsewhere in certain genes (21). The general transcription factors perform a broad range of functions (22–24), from identifying promoter sequences during formation of the preinitiation complex (TFIID and TFIIB) to phosphorylating the C-terminal domain of RNA polymerase (TFIIH), which appears to have a role in the transition from initiation to elongation.

Although previous studies have suggested that the Srb/mediator complex plays a role in the response to transcriptional activators (15, 18, 25–27), the functions of individual components of the complex are poorly understood. Myers *et al.* (14) investigated the role of several subunits of this complex in basal and activated transcription by using mutant forms of holoenzyme in a reconstituted *in vitro* system. They then compared their observations with those obtained *in vivo* by using several assays, including genome-wide expression analysis. The study demonstrated that loss of Med2 causes loss of activation by Gal4-VP16, but not Gcn4, *in vitro* and *in vivo*. The implication is that the response to different activators requires different holoenzyme components. This is consistent with a theme that has emerged from previous studies, but a differential requirement has not been demonstrated in a reconstituted *in vitro* system until now.

The *in vitro* system of Myers *et al.* provides a platform for further analysis of the functions of various components of the holoenzyme. However, it would be interesting to know the influence of components of the transcription apparatus that are not included in this system. For example, Gcn5 and TBP-associated factors (TAF_{II}s) have been shown to be necessary for normal levels of Gcn4-stimulated transcription *in vivo* (28–30). Gcn5, $TAF_{II}68$, and $TAF_{II}17$ are all components of the histone acetylase factor SAGA (31). To complicate matters, $TAF_{II}68$ and $TAF_{II}17$ are subunits shared by both SAGA and TFIID, so it is not yet clear how these two factors are involved in Gcn4-dependent transcription. As the list of factors that regulate gene expression in eukaryotes grows longer, the challenge of reconstituting a system that contains all of the regulatory features that operate at a single gene is becoming considerable.

Significant contributions to our understanding of gene regulation have thus far come from the identification of the promoter elements involved in regulation, the discovery of the transcription apparatus and the factors that regulate it, and the demonstration that chromatin and its regulation plays an important role in gene expression. Future progress will almost certainly involve the discovery of additional regulatory factors through genetic and biochemical approaches and the elucida-

The companion to this Commentary begins on page 67.

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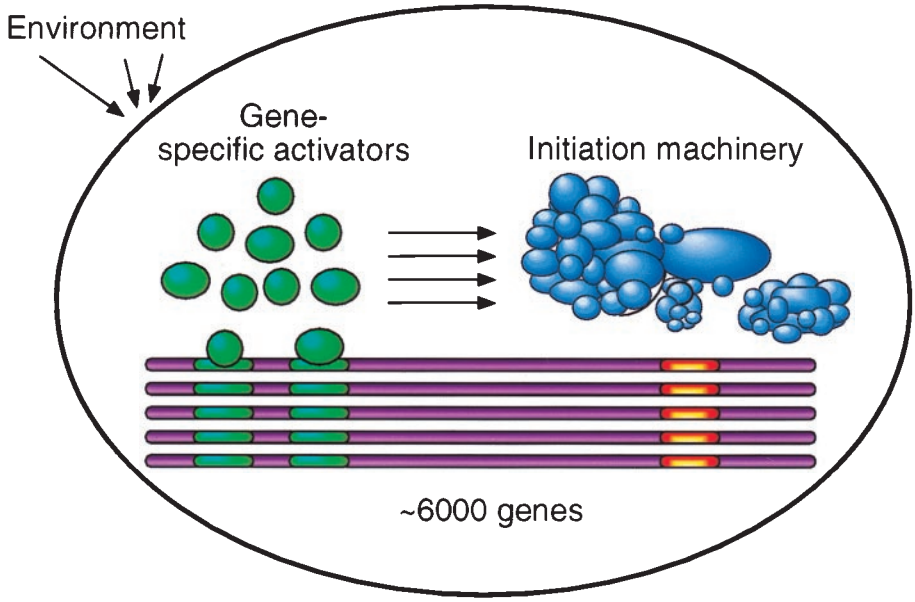

FIG. 1. The transcriptional regulatory problem in yeast. A collaboration among activators, the initiation apparatus, chromatin, and its modifying enzymes is necessary to achieve appropriate transcriptional regulation of all 6,200 yeast genes.

tion of their mechanisms of action using *in vitro* systems reconstituted with purified components. It seems likely, however, that genome-wide expression analysis will provide the most significant new dimension to the field in the immediate future.

Knowledge of the subset of the 6,200 yeast genes that requires the function of each of the components of the transcription apparatus can now be obtained with DNA array technology. This information will produce the framework necessary for further developing *in vitro* systems designed to decipher the regulatory mechanisms that operate at each promoter. Comparison of the expression profiles of cells containing a single mutant component with their wild-type counterparts reveals the set of genes that requires the function of that component. The interpretation of data obtained by this approach is limited by our ability to discriminate between the direct consequences of transcription factor inactivation and the variety of secondary effects that might be caused by the mutation. Tools are being developed to identify the direct consequences of factor inactivation in DNA array experiments (10), but *in vitro* transcription systems reconstituted with the appropriate factors will be necessary to fully understand the regulatory mechanisms at work at specific genes.

A rich history of genetics and biochemistry, together with a complete genome sequence and high density array technology, has set the stage for fully dissecting the complex transcriptional regulatory mechanisms in yeast cells. The recent reconstitution of mammalian systems with RNA polymerase II holoenzymes (32–40), and the anticipated use of high density arrays containing all mammalian genes as these genomes are sequenced, should allow investigators to pursue similar tactics to decipher the much more complex regulatory problems found in mammals.

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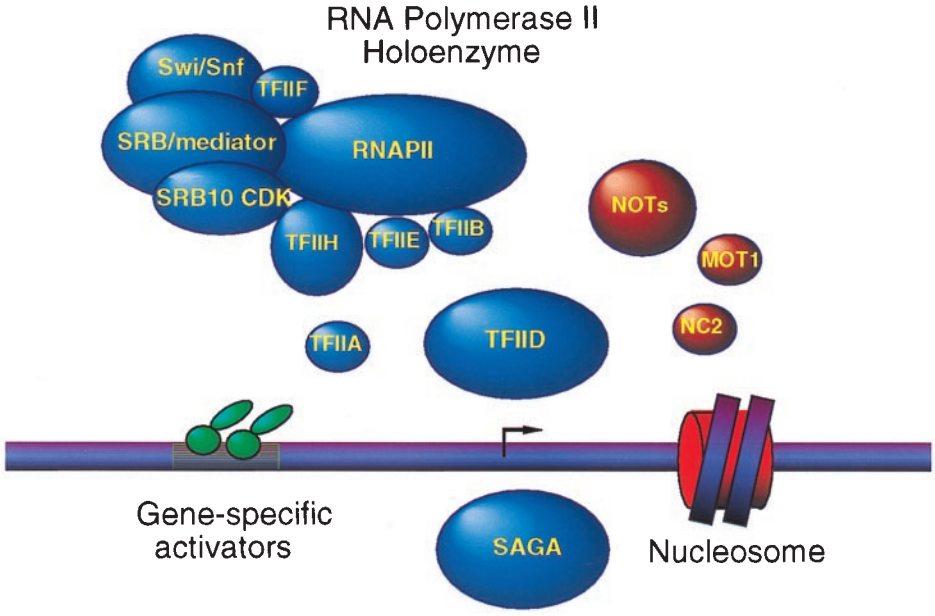

FIG. 2. Model of RNA polymerase II transcription initiation machinery.

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