Annu. Rev. Genet. 2000. 34:77–137


# TRANSCRIPTION OF EUKARYOTIC PROTEIN-CODING GENES

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**Key Words** gene expression, RNA polymerase II holoenzyme, Srb/Mediator complex, chromatin modification, activation and repression

■ **Abstract** The past decade has seen an explosive increase in information about regulation of eukaryotic gene transcription, especially for protein-coding genes. The most striking advances in our knowledge of transcriptional regulation involve the chromatin template, the large complexes recruited by transcriptional activators that regulate chromatin structure and the transcription apparatus, the holoenzyme forms of RNA polymerase II involved in initiation and elongation, and the mechanisms that link mRNA processing with its synthesis. We describe here the major advances in these areas, with particular emphasis on the modular complexes associated with RNA polymerase II that are targeted by activators and other regulators of mRNA biosynthesis.

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0066-4197/00/1215-0077$14.00 

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## INTRODUCTION

The purification of the nuclear RNA polymerases in the 1960s provided the foundation for efforts to understand the regulation of eukaryotic gene expression. Three distinct DNA-dependent nuclear RNA polymerases were purified based on their ability to synthesize a polyribonucleotide copy of a calf thymus DNA template (482). These purified RNA polymerases were incapable of initiating transcription selectively at promoters in vitro (reviewed in 85, 506). Basal or general transcription factors (GTFs) that reconstituted efficient selective initiation by purified RNA polymerase II were identified by fractionation of cellular extracts (reviewed in 113, 211, 424, 473, 480). Purified RNA polymerase II and GTFs alone, however, did not fully reconstitute the response to transcriptional regulators observed in vivo, suggesting that an additional apparatus is necessary for gene regulation in living cells.

Knowledge that the DNA template is packaged into chromatin in vivo prompted genetic, biochemical, and structural studies to understand the roles of nucleosomes and nucleosome-modifying machinery in gene regulation. Promoters for protein-coding genes contain sites bound by transcriptional activators, and activators recruit both chromatin-modifying factors and the transcriptional machinery (reviewed in 303, 461, 493, 533) (Figure 1, see color plate). The importance of chromatin modification in gene expression was emphasized by the discovery that chromatin-modifying enzymes are components of multiple complexes involved in transcription initiation and elongation. These factors act by chemically modifying nucleosomes, as is the case with the Gcn5 acetyltransferase of the SAGA complex, or through noncovalent modifications, as in the case with the Swi/Snf complex (reviewed in 34, 56, 73, 245, 248, 291, 304, 314, 391, 580, 587, 615, 620).

The RNA polymerase II transcription apparatus recruited to promoters and the form of polymerase engaged in elongation are both considerably more complex than previously imagined. Transcriptional activators recruit RNA polymerase II to promoters in a holoenzyme form consisting of GTFs and a multiprotein complex called the Srb/Mediator (reviewed in 39, 42, 197, 212, 302, 342, 369a). Actively

elongating RNA polymerase II molecules are associated with elongation factors (reviewed in 475, 513, 515, 516, 573). Protein complexes involved in RNA capping, polyadenylation and perhaps splicing can also associate with RNA polymerase II (reviewed in 37, 116, 661).

The convergence of information from biochemical, genetic, genomic, and structural studies has greatly increased our understanding of gene regulation in eukaryotes during the past decade. We review here fundamental aspects of gene regulation, focusing on advances in our understanding of the chromatin template, activation and repression, the complexes that regulate chromatin structure, and RNA polymerase II and its initiation, elongation, and processing cofactors.

## THE CHROMATIN TEMPLATE

We briefly review the DNA sequences that are recognized by transcriptional regulators and components of the transcription apparatus. We also describe how DNA is packaged into chromatin, and its implications for gene regulation.

### Promoter and Other DNA Elements

***Basic Promoter Structure*** There are at least three features common to most promoters for protein-coding genes: the transcription start site, the TATA box, and sequences bound by transcriptional regulators (reviewed in 43, 413, 522, 531). The core promoter element, which consists of the start site and the TATA box, is sufficient for directing transcription initiation by the basal transcription machinery. The sequences bound by transcriptional regulators include Upstream Activating Sequences (UASs), enhancers, Upstream Repressing Sequences (URSs), and silencers.

***Core Promoter Element: TATA and Transcription Initiation Site*** The average core promoter element encompasses approximately 100 bp and contains the transcription initiation site. An AT-rich site called the TATA box is located upstream of the start site; its location is 25 to 30 bp upstream in higher eukaryotes and 40 to 120 bp upstream in yeast (reviewed in 531). The TATA box is the binding site for the TATA-binding protein (TBP). Although a canonical sequence can be derived for TATA boxes, TBP can bind and function at a broad range of sequences, making it difficult to identify genuine TBP-binding sites from sequence alone (117, 210, 338, 520, 613).

In some genes, the transcription initiation site includes an initiator (Inr) element (521, 522), defined here as an element encompassing the transcription start site that binds regulatory factors. Various factors can bind to Inr elements, and these may facilitate recruitment of the transcription apparatus (14, 77, 274, 485, 486, 575, 583).

Core promoters can contain TATA and Inr elements (composite), either element alone (TATA- or Inr-directed), or neither element (null) (413). Composite

promoters are found primarily in viral genes; most cellular class II genes contain TATA-directed promoters and a more limited number contain Inr-directed promoters. The null promoters often have multiple transcription start sites, suggestive of imprecise initiation (180, 251, 357).

Although core promoter elements are fundamental for binding of the general transcription apparatus, both the composition and context of the sequence can influence transcriptional regulation. Experiments that exchange TATA boxes and Inr elements reveal that the composition of core promoters can mediate lineage-specific (145, 174), temporal (216), and spatial regulation of gene expression (503).

***Upstream Activating Sequences and Enhancers*** Transcriptional activators bind to sequences that have been termed UASs or enhancers (reviewed in 43, 389, 531). The term UAS is typically used to describe elements bound by activators that influence transcription from nearby start sites. Enhancers are clusters of DNA-binding sites for transcriptional regulators that influence transcription independent of their orientation and at distances as great as 85 kb from the start site (reviewed in 43).

***Upstream Repressing Sequences*** DNA elements bound by sequence-specific gene repressors are called URSs. URS-bound factors inhibit transcription through various mechanisms, including interfering with activator binding, preventing recruitment of the transcription apparatus by the activator, and modifying chromatin structure (reviewed in 215, 264, 296, 368, 418, 447, 531, 534).

***Silencers*** Classical silencers were defined as sequence elements that can repress promoter activity in an orientation- and position-independent fashion (reviewed in 418). The best-characterized examples are involved in silencing of the mating-type genes at the HMR and HML loci in *Saccharomyces cerevisiae*. Two DNA elements, E and I, contain binding sites for Rap1, Abf1, and the origin recognition complex and are essential for silencing that requires Sir proteins and histones H3 and H4 (reviewed in 204, 352, 362). The silencer acts in a distance- and orientation-independent manner. The DNA of the silent domain is thought to be transcriptionally repressed due to the interactions of histone and silencing proteins that cooperatively coat the region and possibly to targeted deacetylation (244).

In higher eukaryotes, a CpG dinucleotide motif has been implicated in silencing mediated by methylation (reviewed in 8, 313, 409, 470).

***Other Elements*** Locus control regions (LCRs) are similar to enhancers in that they consist of multiple activator binding sites, but are often complex arrangements of multiple regulatory elements (reviewed in 165, 199, 337). The two elements differ in that classical enhancers are orientation- and distance-independent, yet their effect can depend on the site of integration into native chromatin, apparently

because the effects of chromatin structure can dominate the function of the enhancer. In contrast, LCRs stimulate transcription independent of their site of integration into native chromatin, although their effects are limited by orientation and distance (reviewed in 61, 149, 165, 199, 337).

Enhancers, LCRs, and silencers can increase or decrease expression of multiple genes within regions of the genome. Additional DNA elements can limit the effects of these regulatory elements, thus subdividing chromosomes into active and inactive regions. Insulators block enhancer function and barriers block the spread of heterochromatin-like silenced domains (reviewed in 32, 33, 537).

## Chromatin Structure

The compaction of DNA that occurs through packaging is necessary to fit into the limiting confines of the nucleus. DNA is packaged into a nucleoprotein complex known as chromatin, consisting of a 2:1 mass ratio of protein to DNA.

***Nucleosomes*** The fundamental repeating unit of chromatin is the nucleosome, which is generally comprised of 146 bp of DNA wrapped 1.65 turns around an octamer of histone molecules, the linker DNA between adjacent histone octamers, and members of a class of linker histones that bind the linker DNA and nucleosome core (305, 666). The co-crystal structure of a histone octamer-DNA complex shows that the histones form a rough cylinder comprised of two heterodimers of histones H3 and H4, flanked by two heterodimers of histones H2A and H2B (358). The crystal structure revealed three features of the nucleosome with implications for gene expression. First, DNA wraps tightly around the core particle and is held in place via multiple interactions between histones and the phosphate backbone or deoxyribose moieties. Second, the arrangement of DNA around the core allows for interactions between the amino-terminal tails of histones and adjacent nucleosome particles. Third, the DNA wrapping about the nucleosome is irregular, providing a degree of flexibility or instability in the structure that may play a role during localized perturbation of histone-DNA interactions critical for gene expression (305, 358, 359).

The four histone subunits that compose the octamer are among the best-conserved proteins in eukaryotes. There are variants reported for a number of histone subunits that appear to have roles in gene-specific transcription. The presence of these variants correlates with induced gene expression in proliferating cells (76), and altered gene expression patterns in chicken (547). In one case, the regions of the variant required for function have been mapped to regions of the histone that are buried in the nucleosome core, suggesting that the alternative structures may influence general nucleosome stability (106). The packaging of promoters into chromatin has been commonly regarded as a general deterrent to transcription (reviewed in 201, 439, 469, 615). Early in vitro experiments with bacterial and eukaryotic polymerases and chromatinized templates demonstrated that nucleosomes could inhibit transcription initiation (295, 354),

whereas preincubation with the transcription apparatus prior to nucleosome formation allowed transcription to occur (294, 617). In vivo experiments also supported this model as depletion of histone subunits in yeast led to increased transcription of certain genes (138, 213, 214, 287, 624). The combination of tight histone-DNA contacts and the organization of nucleosomes into higher-order structures can restrict access to DNA by proteins involved in transcription. Consistent with this model, disruption of nucleosome structure can enhance binding of activators (121, 318, 431) and components of the transcription apparatus (246).

Nucleosomes can potentiate as well as repress gene expression (105, 139, 499, 510, 624). For example, genome-wide expression analysis in yeast revealed that a reduction of nucleosome density leads to increased expression of some genes and decreased expression of others (624). Several mechanisms have been identified for nucleosome-potentiated gene expression (Figure 2, see color plate). Activation of the mouse mammary tumor virus promoter involves synergistic interactions between multiple transcription factors that occur when their binding sites are appropriately positioned by nucleosomes (92). Estrogen-regulated transcription of the *Xenopus* vitellogenin B1 promoter is potentiated by generation of a nucleosome-dependent loop (499). The mouse transcriptional activator Hnf3 can stably bind its target sequence only when the DNA is packaged into a nucleosome (105). Thus, packaging of DNA into chromatin likely provides a distinct physical context for each promoter, thereby increasing the options available to cells for regulation of specific genes.

***Higher-Order Chromatin Structure*** Nucleosomes are coiled or folded into chromatin fibers (439, 578, 665). Linker histones such as histone H1 have been implicated in this organization (reviewed in 31, 468, 555, 564, 578, 588, 608, 614). The flexible amino-terminal histone tails that protrude from nucleosomes may also promote fiber formation, either through direct contact with nucleosomes or by interactions with linker DNA (158, 392, 502; reviewed in 159, 359, 578).

## ACTIVATION AND REPRESSION

Gene-specific transcriptional activation is generally effected by the binding of transcriptional activators to upstream activating sequences, where they recruit and regulate the activities of chromatin-modifying complexes and the transcription apparatus (Figure 1). Regulation of gene expression generally involves an interplay between activators and repressors.

### Activators and Mechanisms

Activators typically consist of two domains: one that binds specific DNA sequences and one that recruits or stimulates the activity of the transcription apparatus (461,

565). A single activator is frequently used to activate multiple genes in a genome, thus providing a mechanism for coordinate control of those genes. Individual genes can be regulated through the action of multiple activators, thereby providing a mechanism for combinatorial control.

Enhanceosomes are formed by the binding of multiple transcriptional regulators to sites in enhancer DNA. The arrangement of binding sites in the enhancer element, the protein-protein interactions of the complement of activators, and the addition of architectural proteins all contribute to the stability and function of enhanceosomes (60, 183, 284, 554; reviewed in 78, 198, 371). The formation of enhanceosomes has several advantages for gene activation. Depending on the nature or state of the cell, different combinations of regulators can be assembled into an enhanceosome. The assembly of these activators can be cooperative, and thus low concentrations of activators are capable of a large range of transcription activation. The arrangement of multiple activators in a single complex provides the capacity to integrate multiple regulatory inputs into a single output.

***Recruitment of Chromatin Modifying Complexes*** Transcriptional activators can recruit chromatin-modifying complexes such as Swi/Snf and SAGA (PCAF) to promoters (44, 120, 243, 309, 406, 407, 451, 462, 576, 644, 647). The importance of chromatin modification in transcriptional regulation is supported by the observation that histone acetylases are components of many transcriptional coactivators (see section on Chromatin Modification). Compelling evidence for the role of certain activators in recruiting chromatin-modifying complexes in vivo comes from the study of *HO* gene activation in yeast, where the transcriptional activator Swi5 recruits the Swi/Snf chromatin modification complex and the SAGA histone acetylase prior to association of a second activator, SBF, which recruits the transcription initiation apparatus (120).

***Recruitment of Transcription Initiation Apparatus*** Activators bind and thereby recruit the RNA polymerase II–containing transcription initiation apparatus (reviewed in 461). At least two arguments support this model. First, many investigators have observed direct binding between activation domains and components of the transcription machinery in vitro (reviewed in 25, 68, 211, 461, 584). Multiple lines of biochemical and genetic evidence indicate that certain activator targets are physiologically relevant (298, 383, 622). Second, if the role of the activation domain is to bind and recruit the transcription apparatus, then fusions of DNA-binding domains to components of the transcription apparatus should bypass the requirement for an activation domain. This prediction is borne out in experiments with "artificial activators," where the fusion protein can substitute for the genuine activator in vivo (10, 27, 90, 152, 175, 176, 187, 261, 275, 292, 625).

A spectrum of models describes activator-dependent recruitment of the transcription apparatus to promoters (Figure 1). At one extreme, the apparatus may

be recruited factor by factor; activators would presumably affect a rate-limiting step. At the other extreme, most or all of the initiation apparatus is recruited as a single complex; here, activators would only need to bind a single component of the complex. Gene activation at the thousands of promoters in a living cell may involve the entire spectrum of mechanistic possibilities.

The transcription apparatus was initially identified and characterized through chromatographic purification of discrete factors necessary to reconstitute transcription with RNA polymerase II in vitro (reviewed in 113, 211, 424, 473, 480). Given the identification of numerous factors by this process, early models of gene regulation proposed that many proteins and complexes were assembled in a stepwise fashion at promoters (63, 160). Significantly, most of these assays required that transcriptional activity be dependent on added core RNA polymerase II, in part because a number of transcriptional activities contaminated the various chromatographic fractions.

The identification and purification of large transcription-competent complexes containing RNA polymerase II, general transcription factors, and additional proteins necessary for the response to activators suggested that activators can recruit much of the transcription apparatus in one or a few steps (289, 301, 428; reviewed in 197, 302). Several of the yeast and metazoan RNA polymerase II holoenzyme preparations appear to contain most of the transcription initiation apparatus (88, 101, 301, 408, 427, 428, 435, 609). In vivo crosslinking experiments with various yeast mutants have demonstrated that a functional Srb4-containing RNA polymerase II holoenzyme is required for stable binding of TBP to promoters, indicating that formation of a stable initiation apparatus is concerted, and consistent with the possibility that stable assembly of the transcription initiation apparatus at promoters effectively occurs in a single step (317, 340).

The concept that has emerged is that a transcription initiation apparatus that approximates the size and complexity of the ribosome is assembled at promoters under the control of, and with the capacity to respond to, combinations of gene-specific regulators bound to enhancers. The modularity of the transcription initiation apparatus enables the cell to recruit components of this initiation apparatus in multiple steps or, if the apparatus is already fully assembled, in a single concerted step.

***Other Functions for Activators*** Activators can also function by influencing the activity of the transcription apparatus. For example, the HIV-1 transcriptional activator Tat stimulates the processivity of RNA polymerase II (reviewed in 171, 259, 272, 479, 483, 638). Certain activators increase the overall elongation rate of polymerase, possibly by stimulating the rate of promoter escape or polymerase II processivity (45, 57, 308, 639).

Some activators may also stimulate multiple rounds of transcription at highly expressed genes by facilitating reinitiation (532, 650). The mechanisms of such stimulation are unclear but may involve stabilization of elements of the transcription apparatus that remain after promoter escape (209).

## Repressors and Mechanisms

The variety of known transcriptional repressors and mechanisms suggests that negative regulation is of critical importance to cells. The fact that these repressors are evolutionarily conserved supports this view. The repressors can be divided into two classes: general and gene specific.

Many general negative regulators function via interactions with the TATA-binding protein (328). Mot1 represses transcription at a subset of class II genes by binding TBP-DNA complexes and causing dissociation of TBP from the DNA in an ATP-dependent manner (18, 340, 396, 592). NC2 (Dr1/DRAP1) is a general negative regulator of class II and class III gene expression (170, 189, 252, 283, 386, 456). NC2 binds to the basic repeat domain of TBP on promoter DNA and can prevent the RNA polymerase II holoenzyme, or its TFIIB subunit, from assembling into an initiation complex (190, 252, 279, 286, 382, 386). *NOT* genes have been identified in a screen for suppressors of a defect in the Gcn4 transcriptional activator and in additional genetic screens as negative regulators of cell cycle, pheromone response, and filamentous growth genes (71, 109, 110, 166, 253, 326, 395, 416, 471). The protein products of these genes form one or more complexes (21, 36, 110, 348). Biochemical and genetic evidence has linked Not proteins physically and functionally to TBP (109, 110, 327), but their mechanism of action is not yet understood.

Many gene-specific repressors function by binding to activators or by competing for activator binding sites. For example, the chaperone Hsp90 binds to the heat shock transcription activator Hsf1, preventing the formation of Hsf1 trimers required for binding of the heat shock DNA element (667). The Gal80 protein represses the Gal4 activator by binding to a portion of its activation domain (336, 363, 519). Repressor proteins can also compete with activators by binding to overlapping sites in DNA, as is the case for the Acr1 repressor and the ATF/CREB activator (589). Gene-specific repressors also include cofactors such as Ssn6-Tup1 that are recruited to specific promoters by DNA-binding factors and repress via interactions with components of the transcription apparatus or chromatin (reviewed in 79, 596).

Histone deacetylases can repress in a gene- or location-specific manner through their action on chromatin, as discussed in detail below. Histone deacetylase complexes such as the mSin3A complex or NuRD are targeted to regions of DNA by a number of mechanisms (reviewed in 19, 296). These deacetylase complexes can be recruited directly by DNA-binding proteins or by co-repressors such as N-Cor, SMRT, Rb, and Groucho, which are in turn recruited by specific DNA-binding activators. Deacetylase activity is also linked to repression mediated by methylated DNA. The mSin3A complex can interact with the methylated DNA-binding protein, MeCP2, and repression mediated by MeCP2 requires this deacetylase activity (265, 405). NuRD contains or interacts with two proteins, MBD3 and MBD2, that have methyl-binding domains similar to MeCP2. Interactions between MBD2 and NuRD can direct the deacetylase complex to methylated DNA (659).

### Interplay Between Activation and Repression

Molecular genetic studies in yeast indicate that regulated gene expression involves a balance of positive and negative regulators in vivo. In at least two instances, genetic selections have demonstrated that a deficiency in a positive factor or DNA element can be compensated by the loss of function of any one of several negative regulators (327, 456, 457). In one case, suppressors of a temperature-sensitive mutation in the *SRB4* gene were isolated; Srb4 protein is an essential component of the RNA polymerase II holoenzyme and is a target of at least one transcriptional activator (298, 557). The suppressors included partial loss-of-function mutations in the negative regulators NC2 and the Not complex (327). In another selection, suppressors of a UAS deletion mutation in the *SUC2* gene were isolated; these suppressors included loss-of-function mutations in NC2, Mot1, and histone H3 (456, 457). These data indicate that expression of specific genes can occur in the absence of fully functional activators or coactivators when the function of certain repressors is compromised.

The argument for an active interplay between positive and negative regulators in vivo is further strengthened by the fact that defects in positive regulators for transcription can compensate for loss-of-function mutations in negative regulators of transcription. Defects in either subunit of NC2 can be rescued by loss-of-function mutations in the Sin4 subunit of the mediator (282). Additional genetic evidence suggests that similar balances exist between negative regulators (Nots, Mot1, and NC2) and positive factors (SAGA acetyltransferase complex and TFIIA) (108, 331, 364, 627).

## CHROMATIN MODIFICATION

The packaging of DNA into nucleosomes and then into higher-order structures has been regarded as generally repressive of transcription (reviewed in 201, 439, 469, 615). It now appears that packaging of DNA into nucleosomes can repress or potentiate gene expression, depending on the requirements of the promoter (see section on The Chromatin Template). Higher-order chromatin structure involves nucleosome-nucleosome contacts mediated, in part, by the N-terminal tails of histones (392, 566, 665). An excellent correlation exists between acetylation of the N-terminal tails of histones, disruption of higher-order chromatin structure, and transcriptional activity. Similarly, there is a good correlation between histone deacetylation, the formation of higher-order structure, and repression of transcription (reviewed in 129, 305, 526).

### Covalent Modifications of Histones

Histones are modified by acetylation, phosphorylation, methylation, and ubiquitination (Table 1). In many instances, the precise sites of modifications have been identified (Figure 3). For example, histone H3 is primarily acetylated at lysines

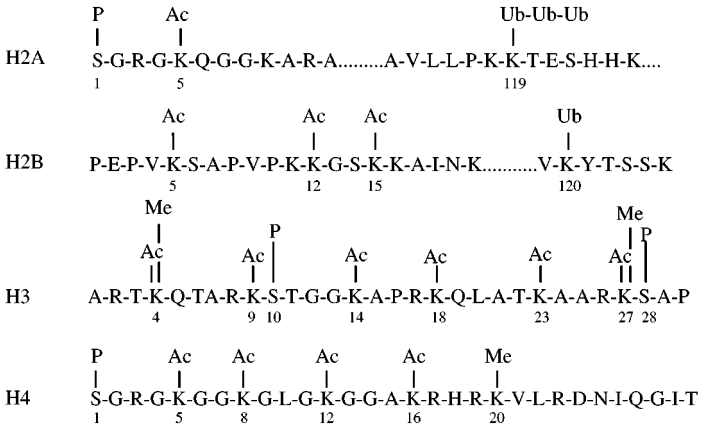


**Figure 3** Sites of covalent modifications of histones. The sites for acetylation (Ac), phosphorylation (P), methylation (Me), and ubiquitination (Ub) in core histones (modified from 526).

9, 14, 18, and 23, phosphorylated at serine 10, and methylated at lysines 9 and 27 (526). Combinatorial modifications to histone tails may serve as a code to instruct cellular actions on the DNA template such as assembly, mitosis, transcription, or replication (529).

Histone acetylation is the best understood of the histone modifications, both in terms of the residues affected and the consequences for transcriptional activity. Several lysines on the N-terminal tail of each of the core histones can be reversibly acetylated. Considerable evidence supports a positive link between acetylation of histone tails and transcriptional activity. Hyperacetylated histones are stably associated with transcriptionally active domains and more accessible chromatin structure, whereas hypoacetylated histones are enriched in regions that are transcriptionally silent (219, 220, 598, 654). The enzymes involved in histone acetylation and deacetylation are described in detail in the next section.

The relationship between histone acetylation and transcriptional activity may involve more than one mechanism. Since acetylation disrupts higher-order chromatin structure (173, 567, 597, reviewed in 526), it may provide greater access to DNA sequences for the transcription apparatus and its regulators. Acetylation of the histone tail may disrupt nucleosome structure by neutralizing positively charged lysines, thus decreasing their affinity for DNA or neighboring nucleosomes (201, 359). Acetylation may also influence transcription by promoting or suppressing interactions with specific transcription factors (129, 615, 616).

Histone tails are also modified by phosphorylation. Phosphorylation of histones H1 and H3 has been implicated in chromosome condensation during mitosis (50, 307, 606, 607). Phosphorylation of H3 has also been linked to increased transcriptional activity. For example, histone H3 phosphorylation correlates with

**TABLE 1** Enzymes that covalently modify histones

| Protein | Organism | Comment |
|---|---|---|
| Acetylation | | |
| p55 | *T. thermophila* | Gcn5-like |
| p80 | *T. thermophila* | Preferentially acetylates H4 |
| Hat1 | *S. cerevisiae* | B-type HAT, acetylates H4, believed to function in cytoplasm |
| yGcn5 | *S. cerevisiae* | Transcriptional coactivator |
| Hpa2 | *S. cerevisiae* | Gcn5-like, similar to yeast Hpa3 |
| Esal | *S. cerevisiae* | MYST family, essential for viability |
| Sas3 | *S. cerevisiae* | MYST family, highly similar to yeast Sas2 |
| Elp3 | *S. cerevisiae* | Associates with elongating RNA polymerase II |
| TAFII145 | *S. cerevisiae* | Subunit of yeast TFIID |
| HatA | *Z. mays* | Two forms, A1 and A2 identified |
| HatB | *Z. mays* | B-type HAT |
| dTAFII230 | *D. melanogaster* | Subunit of *Drosophila* TFIID |
| xHat1 | *X. laevis* | Acetylates free H4 |
| hTAFII250 | *H. sapiens* | Subunit of human TFIID |
| TFIIIC220 | *H. sapiens* | Subunit of human TFIIIC |
| TFIIIC110 | *H. sapiens* | Subunit of human TFIIIC |
| TFIIIC90 | *H. sapiens* | Subunit of human TFIIIC |
| hHat1 | *H. sapiens* | Acetylates free H4 |
| hGcn5-L | *H. sapiens* | Gcn5-like, transcriptional coactivator |
| PCAF | *H. sapiens* | Gcn5-like, transcriptional coactivator |
| CBP/p300 | *H. sapiens* | Transcriptional coactivator |
| SRC-1 | *H. sapiens* | Nuclear receptor coactivator, family includes TIF2/GRIP1 |
| ACTR | *H. sapiens* | SRC family, isoforms include RAC3, TRAM1, AIB1, p/CIP |
| HBO1 | *H. sapiens* | MYST family, binds origin recognition complex |
| MORF | *H. sapiens* | MYST family, similar to human MOZ and *Drosophila* MOF |
| Tip60 | *H. sapiens* | MYST family, HIV-1-Tat interacting protein |
| Deacetylation | | |
| Rpd3 | *S. cerevisiae* | Involved in repression |
| Hda1 | *S. cerevisiae* | Deletions affect acetylation level in vivo |
| Hos3 | *S. cerevisiae* | Forms homodimer that has intrinsic activity |
| ySir2 | *S. cerevisiae* | Involved in aging, NAD+ dependent |
| HD1A | *Z. mays* | |
| zmRpd3 | *Z. mays* | HDIB-1 |
| HD2 | *Z. mays* | Nucleolar phosphoprotein |
| dRpd3 | *D. melanogaster* | Affects silencing and position effect variegation |
| dHDAC3 | *D. melanogaster* | |
| xRpd3 | *X. laevis* | Associates with multiple complexes |

**TABLE 1** (*Continued*)

| Protein | Organism | Comment |
|---|---|---|
| mHda2 | *M. musculus* | Contains two HDAC domains |
| mHDAC1 | *M. musculus* | |
| mHDAC2 | *M. musculus* | |
| mHDAC3 | *M. musculus* | |
| mHDAC5 | *M. musculus* | Previously mHdal |
| HDAC1 | *H. sapiens* | Recruited by many DNA-binding regulators w/HDAC2 |
| HDAC2 | *H. sapiens* | Recruited by many DNA-binding regulators w/HDAC1 |
| HDAC3 | *H. sapiens* | Can associate with HDAC4 and HDAC5 |
| HDAC4 | *H. sapiens* | Cellular localization may regulate activity |
| HDAC5 | *H. sapiens* | Can bind N-CoR/SMRT corepressors |
| HDAC6 | *H. sapiens* | Contains two HDAC domains |
| HDAC7 | *H. sapines* | Can interact with SMRT corepressor |
| HDAC8 | *H. sapiens* | Tissue-specific expression differs from other HDACs |
| hSir2 | *H. sapiens* | Involved in aging, NAD+ dependent |
| Phosphorylation | | |
| JIL-1 | *D. melanogaster* | Associated with dosage compensation |
| Rsk-2 | *H. sapiens* | Phosphorylates H3 Ser 10, implicated in Coffin-Lowry disease |
| MSK1 | *H. sapiens* | Phosphorylates H3 Ser 10 |
| Methylation | | |
| CARM1 | *H. sapiens* | Arginine-specific, histone H3 methyltransferase activity |

activation of SV40 immediate early genes (80, 365, 558). Several histone kinases are associated with transcriptionally active states. Cells deficient in the Rsk-2 H3 kinase display altered transcription activation patterns (130, 492). The Msk1 kinase, which is activated by growth factor, phosphorylates histone H3 (558). The Jil-1 kinase, which is involved in dosage compensation in Drosophila, also phosphorylates histone H3 (263).

Histones can be reversibly ubiquitinated, primarily on histone H2A but also on H2B and H3, and these ubiquitinated histones are associated with transcriptionally active DNA. Ubiquitination is dependent on ongoing transcription (128), possibly because transcription is required to expose the regions of histones that are typically ubiquitinated (128). The addition of ubiquitin may serve to disrupt nucleosome structure (526), but a regulatory role for histone ubiquitination in transcription is not yet firmly established.

Histones H2B, H3, and H4 can also be methylated, but the effects of histone methylation on transcription remain poorly understood. Methylation is associated

with acetylated isoforms of H3 and H4 (7, 223, 224), suggesting that methylation, acetylation, and transcriptional activation may correlate in some cases. This idea is supported by evidence that a histone methyltransferase, CARM1, is required for ligand-dependent activation of some nuclear hormone receptors (93).

## Histone Acetyltransferases

***Linking Transcription and Histone Acetylation*** The key discovery linking transcription and acetylation was the identification of a *Tetrahymena* protein, p55, which had histone acetyltransferase (HAT) activity and was related to a yeast protein, Gcn5 (59). As Gcn5 had previously been identified as a transcriptional cofactor (372), this finding provided a functional connection between acetylases and transcriptional activation. Chromatin in the region of Gcn5-dependent promoters shows increased acetylation of histone subunits upon stimulation of transcription by Gcn5 (316). Mutations in the HAT catalytic domain affected both the ability to activate transcription and the acetylation of promoter-proximal histones (316, 601). Now that a large number of HATs have been described, the fact that many are components of previously identified transcriptional coactivators underscores the functional link between acetylation and activation.

***HATs Are Associated with Multisubunit Complexes*** Biochemical fractionation revealed that Gcn5 can be purified in multiple complexes capable of acetylating histones in the context of the nucleosome octamer. One of these is SAGA, named for the Spt, Ada, and Gcn5 Acetyltransferase components (193). The Spt and Ada genes were previously identified as regulators involved in start site selection and transcriptional activation (reviewed in 611). SAGA shares a number of subunits with the TFIID general transcription factor (192). Gcn5 exists in at least one other complex called the ADA complex (142). Recombinant Gcn5 can acetylate histones in vitro, but not nucleosomes (315, 637). As part of the multiprotein complexes, Gcn5 gains the ability to acetylate nucleosomes and the ability to acetylate additional lysines (194).

Numerous acetyltransferases and acetyltransferase complexes have been identified (Table 2). Histone acetyltransferases (HATs) can be grouped by cellular location into two classes (58). Type A HATs are localized in nuclei and most likely acetylate nuclear factors. In contrast, type B HATs are cytoplasmic and are believed to acetylate newly synthesized histones as part of the process of histone assembly (249, 293, 353, 441).

***Coactivators with HATs*** Two yeast and three human complexes have been purified that contain Gcn5-like histone acetyltransferases (54, 142, 192, 377, 419). Given the observation that the substrate specificity of Gcn5 can be influenced by its association with other proteins, each of these complexes may have distinct roles. However, it is also possible that the multiple Gcn-5 complexes are derived from a single large complex in vivo that is torn asunder during column chromatography.

**TABLE 2** HAT complexes

| HAT complexes | HAT Subunit | Organism | Complex activity |
|---|---|---|---|
| Hat1 complex | Hat1 | *S. cerevisiae* | Acetylates free H4, not nucleosomal |
| ADA | Gcn5 | *S. cerevisiae* | Acetylates nucleosomal H3 |
| SAGA | Gcn5 | *S. cerevisiae* | Acetylates nucleosomal H3 and H2B<br>Interacts with activators and transcription machinery |
| NuA3 | unknown | *S. cerevisiae* | Acetylates nucleosomal H3 |
| NuA4 | Esa1 | *S. cerevisiae* | Acetylates nucleosomal H4 and H2A |
| yTFIID | TAFII145 | *S. cerevisiae* | Acetylates free H3 and H4<br>Core promoter factor |
| Elongator | Elp3 | *S. cerevisiae* | Acetylates H2A, H2B, H3, H4<br>Associated with elongating RNA polymerase II |
| HatB complex | HatB | *Z. mays* | Acetylates free H4<br>Associates with RbAp-related protein |
| dTFIID | dTAFII230 | *D. melanogaster* | Acetylates free H3 and H4<br>Core promoter factor |
| Hat1 complex | xHat1 | *X. laevis* | Acetylates free H4<br>Associates with RbAp48 and 14-3-3 proteins |
| hHat1 | hHat1 | *H. sapiens* | Acetylates free H4<br>Associates with RbAp46/48 |
| hTFIID | hTAFII250 | *H. sapiens* | Acetylates free H3 and H4<br>Core promoter factor |
| TFTC | Gcn5-L | *H. sapiens* | Acetylates nucleosomal H3<br>Can substitute for TFIID in vitro |
| STAGA | Gcn5-L | *H. sapiens* | Likely identical to TFTC |
| PCAF | PCAF | *H. sapiens* | Transcriptional coactivator<br>Interacts with other HAT coactivators<br>Interacts with DNA-binding regulators<br>Acetylates nucleosomal H3<br>Acetylates TFIIF, TFIIE, p53 |
| TFIIIC | p220<br>p110<br>p90 | *H. sapiens* | Acetylates H2A, H3 and H4<br>Core promoter factor for RNA polymerase III |

Many DNA-binding transcription factors, including CREB, p53, and nuclear hormone receptors, bind to a set of coactivators called p300/CBP. p300 and CBP are two highly related proteins that apparently regulate overlapping sets of genes (143, 184). Both p300 and CBP have HAT activity (24, 420) and may function in cooperation with additional coactivators such as PCAF, SRC-1, TIF2/GRIP1, and RAC3/TRAM1/AIB1/PCIP/ACTR (reviewed in 95, 332). Many of these coactivators also contain acetyltransferase activity, indicating that activators can recruit multiple HATs for full stimulation of transcription (94, 525, 637). Recent experiments suggest that although a coactivator complex can contain multiple acetyltransferases, transcription activation may require the activity of only one of these factors at any single promoter (306, 462).

***Targeting of Acetyltransferases*** In general, HAT activity is restricted to gene-specific effects via interactions with DNA-binding activators (Figure 2). For example, recruitment of the SAGA complex to the *HO* promoter is dependent on the transcriptional activator Swi5 (120). SAGA and NuA4 interact with specific subsets of activation domains (243, 406, 576). Experiments in yeast demonstrate that gene-specific targeting via interactions with DNA-binding activators can result in increased acetylation of promoter-bound histones in vitro (243, 576) and transcriptional activation of target genes in vivo (316). In mammalian cells, p300/CBP is targeted by various cellular and viral factors (143, 258) including activators like c-jun and nuclear hormone receptors like Pit-1, MyoD, or CREB (306, 462). As mentioned previously, p300/CBP may serve as a binding platform for the recruitment of additional cofactors, some of which also contain acetyltransferase activity.

A novel acetyltransferase, Elp3, has been identified as a component of yeast Elongator, a complex that tightly associates with elongating RNA polymerase II (612). This physical link between an acetylase and RNA polymerase II might provide a means to modify the acetylation state of nucleosomes in transcribed regions of chromatin (612).

***Histone versus Factor Acetyltransferases*** Histone acetyltransferases can acetylate substrates other than histones (reviewed in 38, 314), including DNA-binding activators such as p53 (203), EKLF (656), GATA-1 (49, 242); architectural proteins like HMG-I or HMG-17 (228, 397); and the general transcription factors TFIIE and TFIIF (250). Acetylation of factors appears to have both positive and negative effects on transcription, in some cases at the same promoters. For example, targeted acetylation of histones at the IFN-beta promoter occurs early during viral infection and requires CBP, consistent with a model where acetylation of histones is required for gene activation. CBP and PCAF also acetylate HMG-I, which is the architectural component of the assembly of activators (enhanceosome) that stimulates IFN-beta transcription. Acetylation of HMG-I reduces its affinity for DNA, destabilizing the enhanceosome and reducing IFN-beta transcription in vitro (440). p53 is another interesting example of factor acetylation. Acetylation by CBP/p300 and PCAF both enhance p53's DNA-binding activity even though

the two factors acetylate different sites on p53. Both sites display increased acetylation upon stimulation of p53 function, suggesting that the combinatorial action of acetyltransferases may be critical for regulation of function (349, 490).

## Histone Deacetylases

***Linking Transcription and Histone Deacetylation*** Histone deacetylases were linked to transcription when the purification and peptide sequencing of histone deacetylases revealed sequence similarities to transcriptional regulators previously identified in yeast. The first histone deacetylase, HDAC1, was purified and cloned based on its ability to bind the deacetylase inhibitor trapoxin (553). HDAC1 proved to be similar to yeast Rpd3, which had been identified as a transcriptional regulator through a genetic selection (585). In separate work, fractionation of yeast extracts identified two distinct deacetylation activities, HDA and HDB (488). The catalytic subunits of these complexes were identified as the Hda1 (HDA complex) and Rpd3 (HDB complex) proteins (488). Null mutations in HDA1 or RPD3 result in hyperacetylation of the N-terminal tails of histones H3 and H4 (488). Deacetylases have been identified as common components of corepressors recruited by diverse DNA-binding regulators, indicating that deacetylases play a general role in repression of gene expression.

***HDACs Are Associated with Multiprotein Complexes*** Like acetyltransferases, deacetylases are found in multiprotein complexes. Two corepressor complexes, Sin3 and NuRD (which is highly similar or identical to NURD, NRD, and Mi-2 complex), have been characterized thus far. In mammalian cells, both complexes contain a core of HDAC1, HDAC2, and histone binding proteins RbAP46 and RbAP48 (659; reviewed in 19, 296). The assembly of the core HDAC into larger complexes affects both the substrate specificity and corepressor interactions of the core HDAC complex (319, 660).

Sin3 complexes are characterized by the presence of the corepressor Sin3. In mammalian cells, there are two isoforms of Sin3, mSin3A and mSin3B. Both are similar to the yeast transcription regulator Sin3 (20). The yeast *SIN3* gene functions in the same genetic pathway as *RPD3* (528, 585, 586), providing an early indicator that deacetylation might explain Sin3-mediated repression. mSin3A was originally identified as a corepressor for the DNA-binding repressor Mad-Max (20, 501). Several groups have now demonstrated that deacetylases associate with Sin3 in yeast and mammalian systems and that deacetylation is essential for full Sin3-mediated repression (3, 218, 221 320, 402, 657).

In mammalian systems, HDAC1 and HDAC2 also exist in the NuRD complex (563, 593, 629, 658). NuRD is characterized by the presence of two closely related proteins, CHD3 and CHD4 (also known as Mi-2$\alpha$ and Mi-2$\beta$, respectively). CHD3/CHD4 are similar to the Swi/Snf family of DNA-dependent ATPases that remodel chromatin, and as might be expected, NuRD is capable of remodeling nucleosomes in an ATP-dependent fashion. The deacetylase activity of NuRD is stimulated by the addition of ATP, suggesting that the remodeling ability of

the complex may be used in vivo to facilitate access to histone tails in complex chromatin structures (563, 593, 629, 658).

A number of HDACs have now been identified (Table 1). In yeast, there are at least five deacetylases, Rpd3, Hda1, Hos1, Hos2, and Hos3, that are grouped into class I (Rpd3-like) and class II (Hda1-like) families. In humans, eight deacetylases have been identified—the class I members HDAC1, HDAC2, HDAC3, and HDAC8 (29, 148, 239, 634, 635, 653) and the class II members HDAC4, HDAC5, HDAC6, and HDAC7 (200, 271). More recently, the Sir2 protein, which is involved in gene silencing, was demonstrated to be an NAD-dependent histone deacetylase, suggesting that there is additional diversity in the deacetylases of eukaryotic cells, and linking deacetylation to silencing and aging in yeast and mammalian cells (244). A fourth type of deacetylase has been isolated in maize, but it is unclear if this deacetylase has roles in transcription (361).

***Targeting of Deacetylases*** mSin3A complexes are capable of diverse interactions with DNA-binding regulators and corepressors, reflecting the diverse mechanisms employed to direct deacetylase activity to specific promoters or sets of promoters (Figure 4) (reviewed in 19, 296). The deacetylase complex can be recruited directly by DNA-binding proteins, including Mad, Ume6, Ski, Ikaros, and p53. mSin3A complexes can also be recruited via interactions with corepressors for nuclear hormone receptors, most notably N-Cor and SMRT. mSin3A

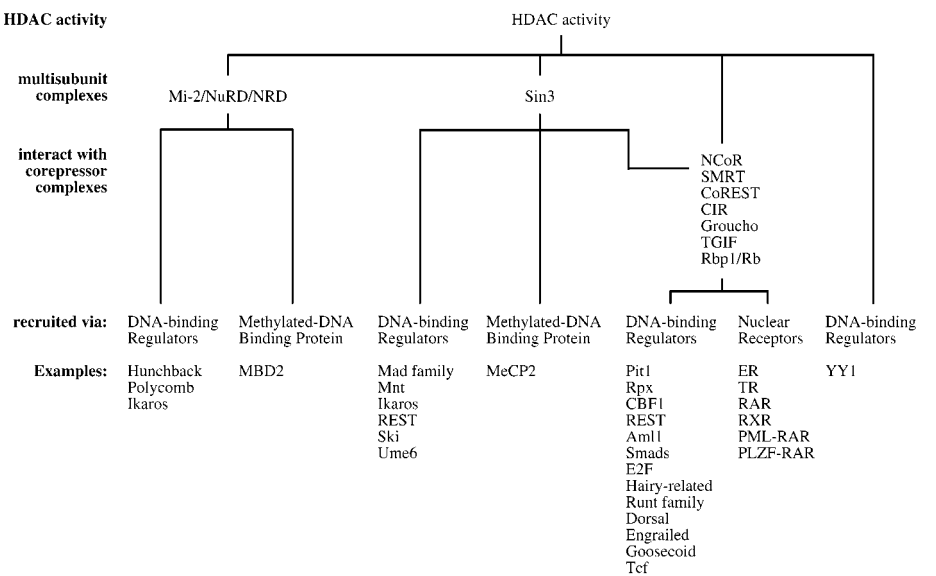


**Figure 4** Recruitment of HDAC activity. HDAC activity can be targeted to specific genes or regions through interactions with diverse corepressors and DNA-binding regulators.

complexes also associate with additional corepressors such as Rb and Groucho, which are in turn recruited by specific DNA-binding activators.

The NuRD/NRD/Mi-2 complex is also likely to be recruited to promoters via interactions with DNA-binding regulators (reviewed in 19, 296). NuRD interacts with the Hunchback and Polycomb repressors in *Drosophila*, and the Ikaros transcription factor in T cells.

Recruitment of deacetylase activity to particular regions of the genome has been linked to interactions between deacetylases and proteins that bind methylated DNA. Transcriptionally silenced regions are associated with both hypoacetylated and hypermethylated DNA (41, 266, 409, 470). Methylation-mediated repression requires the presence of nucleosomal structure (69, 273), suggesting a link between chromatin, methylation state, and repression. The mSin3 complex can interact with the methylated DNA-binding protein MeCP2, and repression mediated by MeCP2 requires deacetylase activity (265, 405). NuRD contains or interacts with two proteins, MBD3 and MBD2, that have methyl binding domains similar to MeCP2. Interactions between MBD2 and NuRD can direct the deacetylase complex to methylated DNA (659).

## Noncovalent Chromatin Modification

Nucleosomes are subjected to conformational remodeling in addition to covalent modifications (reviewed in 34, 73, 245, 291, 304, 451, 480, 587, 615, 620). Remodeling involves the breaking and reforming of histone-DNA contacts that result in the mobilization of nucleosomes in the chromatin template. Although the precise mechanism of such chromatin remodeling is unknown, several different remodeling complexes have been identified (Table 3), the best studied of which are the Swi/Snf and Rsc complexes from yeast and the NURF, CHRAC, and ACF complexes of *Drosophila.* All of these complexes contain an ATPase subunit that is essential for remodeling activity along with additional subunits that affect regulation, efficiency, and specificity.

Chromatin remodeling factors apparently act by catalyzing fluidity in the position and conformation of nucleosomes (reviewed in 291). They are thought to do this by catalyzing the interconversion between various chromatin states via an activated intermediate consisting of the remodeling factor and a nucleosome with weakened histone-DNA contacts. As this model posits that remodeling complexes increase the rate of interconversion between chromatin states, an important implication is that the action of remodeling complexes does not specify whether the resulting chromatin state is positive or negative for transcription. Genome-wide analysis of the effects of loss of the Swi2 remodeling factor in yeast indicates that this remodeling factor has positive roles in transcription at some genes and negative roles at other genes (236, 536).

***Diversity of Functions of Chromatin Remodeling Factors*** The number and diversity of chromatin remodeling factors appear to reflect differences in their function. There are two main families of ATPase subunits, each with distinct

**TABLE 3** Chromatin remodeling factors

| Complex | Organism | Activities of complex | Subunits | Features | Size |
|---|---|---|---|---|---|
| SWI/SNF Family | | | | | |
| Swi/Snf | *S. cerevisiae* | Chromatin remodeling activity | Swi1 | | 148 kDa |
| | | | Swi2/Snf2 | ATPase, not essential | 194 kDa |
| | | | Swi3 | Similar to RSC8 | 93 kDa |
| | | | Snf5/Swi10 | | 103 kDa |
| | | | Snf6 | | 37 kDa |
| | | | Snf11 | | 19 kDa |
| | | | Snf12 | Swp73, similar to RSC6 | 73 kDa |
| | | | Swp82 | | ~82 kDa |
| | | | Swp59 | Arp9, actin-related protein | 53 kDa |
| | | | Swp61 | Arp7, actin-related protein | 54 kDa |
| | | | Swp29 | Subunit of TFIIF and TFIID | 27 kDa |
| RSC | *S. cerevisiae* | Chromatin remodeling activity | Sth1 | ATPase, essential | 157 kDa |
| | | | RSC1 | | 107 kDa |
| | | | RSC2 | | 102 kDa |
| | | | RSC3 | | 90 kDa |
| | | | RSC4 | | 72 kDa |
| | | | RSC5 | | ~65 kDa |
| | | | RSC6 | Similar to SNF12 | 54 kDa |
| | | | RSC7 | | ~60 kDa |
| | | | RSC8 | Similar to SW13 | 63 kDa |
| | | | RSC9 | | ~55 kDa |
| | | | RSC10 | | ~55 kDa |
| | | | RSC11 | Arp7, actin-related protein | 54 kDa |
| | | | RSC12 | Arp9, actin-related protein | 53 kDa |
| | | | RSC13 | | ~27 kDa |
| | | | RSC14 | | ~23 kDa |
| | | | RSC15 | | ~23 kDa |
| | | | Sfh1 | Similar to SNF5 | 49 kDa |
| hSwi/Snf | *H. sapiens* | Chromatin remodeling activity | BAF250 | | ~250 kDa |
| | | | Brg1 or hBrm | ATPase, essential<br>ATPase, not essential | ~190 kDa |
| | | | BAF170 | | ~170 kDa |
| | | | BAF155 | Similar to Swi3 | ~155 kDa |
| | | | BAF110 | Similar to Swi3 | ~110 kDa |
| | | | BAF60a | Similar to Snf12 | ~60 kDa |
| | | | or BAF60b | Tissue-specific BAF60 | ~60 kDa |
| | | | or BAF60c | Tissue-specific BAF60 | ~60 kDa |
| | | | BAF57 | HMG domain, kinesin-like region | ~57 kDa |
| | | | BAF53 | Actin-related protein | ~53 kDa |
| | | | BAF47 | INI1/hSnf5, similar to Snf5 | ~47 kDa |
| dSwi/Snf | *D. melanogaster* | Chromatin remodeling activity | Brahma | ATPase, essential | ~190 kDa |
| | | | BAP155 | Similar to Swi3 | ~155 kDa |
| | | | BAP111 | | ~111 kDa |
| | | | BAP74 | | ~74 kDa |
| | | | BAP60 | Similar to Snf 12 | ~60 kDa |
| | | | BAP55 | Actin-related protein | ~55 kDa |
| | | | BAP47 | | ~47 kDa |
| | | | BAP45 | SNR1, similar to Snf5 | ~45 kDa |
| ISWI Family | | | | | |
| ISW1 | *S. cerevisiae* | Nucleosome remodeling and spacing activity | Isw1 | ATPase, not essential | 130 kDa |
| | | | p110 | | 110 kDa |
| | | | p105 | | 105 kDa |
| | | | p74 | | 74 kDa |
| ISW2 | *S. cerevisiae* | Nucleosome spacing activity | p140 | | 140 kDa |
| | | | Isw2 | ATPase, not essential | 130 kDa |
| NURF | *D. melanogaster* | Nucleosome spacing activity | NURF-215 | | 215 kDa |
| | | | ISWI | ATPase | 140 kDa |

**TABLE 3** (*Continued*)

| Complex | Organism | Activities of complex | Subunits | Features | Size |
|---|---|---|---|---|---|
| | | | NURF-55 | Homologous to RbAp48 | 55 kDa |
| | | | NURF-38 | Inorganic pyrophosphatase | 38 kDa |
| ACF | *D. melanogaster* | Chromatin assembly, spacing, remodeling activity | ISWI | ATPase | 140 kDa |
| | | | Acf1 | | 185 kDa |
| CHRAC | *D. melanogaster* | Nucleosome remodeling and spacing activity | p175 | | 175 kDa |
| | | | p160 | Topoisomerase II | 160 kDa |
| | | | ISWI | ATPase | 130 kDa |
| | | | p20 | | 20 kDa |
| | | | p18 | | ~18 kDa |
| hACF | *H. sapiens* | Chromatin assembly, remodeling activity | hSnf2h | ATPase | 135 kDa |
| | | | BAZIA | | 190 kDa |
| RSF | *H. sapiens* | Enhances transcription from chromatin templates | p325 | | 325 kDa |
| | | | hSnf2h | ATPase | 135 kDa |

complexes identified in several different organisms (Table 3). Complexes with ATPase subunits from different families appear to have different substrate requirements in vitro. NURF requires histone tails for its activity, whereas Swi/Snf does not (181, 207, 351). Several ISWI complexes are stimulated only by nucleosomal DNA, whereas Swi/Snf is stimulated by nucleosomal as well as naked DNA (121, 323, 570).

The substrate specificities between complexes with Swi/Snf or ISWI suggest potential mechanistic differences among the remodeling factors, but additional functional differences are apparent between complexes with similar or identical ATPase subunits. For example, yeast Rsc complex is essential for viability, whereas Swi/Snf is not, presumably reflective of different roles in vivo (75). Individual ISWI complexes display differences in their activities, most likely reflecting differences in their mechanisms and their functions in vivo (568–570, 582). For instance, multiple ISWI-containing complexes can generate ordered arrays of nucleosomes from irregularly spaced arrays, but the extent of ordering and the spacing between nucleosomes vary with the different complexes, presumably reflecting the differences in their subunits and composition (255, 569, 581).

Like the multisubunit forms of histone acetyltransferases and histone deacetylases, the subunits associated with the ATPase subunits influence the functions of the complex. The recombinant ISWI ATPase subunit can perform several functions characteristic of ISWI-containing complexes, including promoting uniform spacing of nucleosome arrays, remodeling of chromatin in conjunction with the GAGA factor, and repositioning of nucleosomes (118, 321). ISWI in the context of the CHRAC complex displays an altered pattern of nucleosome repositioning (321). Similarly, ISWI in the context of the ACF complex is stimulated in its ability to assemble appropriately spaced nucleosome arrays (256).

***Recruitment of Remodeling Factors*** Swi/Snf is the best characterized remodeling complex, and significant effort has been devoted to identifying how this factor is recruited to promoters. Swi/Snf is likely targeted to specific promoters by DNA-binding activator proteins (Figure 1) (120, 133, 406, 407, 647). In mammalian systems, several steroid receptors interact with components of Swi/Snf, and the glucocorticoid receptor can target ATP-dependent remodeling activities to a mononucleosome (167, 643, 644). While no sequence-specific DNA-binding activity has been identified in Swi/Snf, the complex can bind DNA structures that may contribute to directing Swi/Snf to nucleosomes (1, 415, 465, 602). Swi/Snf may also be targeted to promoters via interactions with the general transcription machinery (102, 408, 609).

## RNA POLYMERASE II AND INITIATION COFACTORS

Transcriptional activators recruit the RNA polymerase II–containing transcription initiation apparatus to promoters of protein-coding genes. The assembled apparatus contains the 12-subunit RNA polymerase II core enzyme, the general transcription factors, and one or more multisubunit complexes called coactivators or mediators. RNA polymerase II holoenzymes that contain most of these components of the initiation apparatus in a single complex have been purified from yeast and mammalian cells, suggesting that much of this apparatus can be recruited to promoters in one step.

The best-defined RNA polymerase II holoenzyme is from yeast and contains RNA polymerase II, a subset of the general transcription factors and the Srb/Mediator complex. The Srb/Mediator complex appears to integrate signals from transcriptional regulators at promoters, and its composition can be remodeled as cells encounter new environments to allow coordinate control of specific sets of genes. Recent studies have revealed that various mammalian coactivators, purified independently by multiple investigators for their ability to reconstitute activated transcription of different genes, are homologues of the yeast Srb/Mediator complex.

### Core RNA Polymerase II

Purified eukaryotic core RNA polymerase II typically has 10 to 12 subunits (Table 4). Core RNA polymerase II is capable of DNA-dependent RNA synthesis in vitro, but is incapable of specific promoter recognition in the absence of additional factors. Yeast and human RNA polymerase II consist of 12 subunits, Rpb1 to Rpb12. The genes encoding the 12 yeast subunits are all required for normal cell growth. Genes for each of the 12 human RNA polymerase II subunits have been isolated and exhibit remarkable structural and functional conservation, as most human subunit genes can functionally substitute for their counterparts in yeast (reviewed in 618).

Eukaryotic RNA polymerase II molecules share several important features with their bacterial counterparts that provide clues to subunit functions. The bacterial RNA polymerases are composed of a specificity factor ($\sigma$), and a three-component core enzyme, structured as a $\beta$, $\beta'$, $\alpha 2$ tetramer. The two largest subunits, Rpb1 and Rpb2, are homologous to the $\beta'$ and $\beta$ bacterial core subunits, respectively. Rpb3 and Rpb11 share a weaker homology with the $\alpha$ bacterial core subunit and can form a Rpb3-Rpb11 heterodimer in vitro (322). The eukaryotic subunit orthologues of these bacterial core enzyme subunits are largely responsible for RNA catalysis (reviewed in 618, 646).

The structure of yeast RNA polymerase II has been solved at 3 angstroms resolution and has revealed several interesting features (122a). Comparison of previously solved structures of prokaryotic RNA polymerase with the structure of eukaryotic RNA polymerase II reveals that the structures of the core subunits of these enzymes are very similar (122a, 655). The two largest subunits, Rpb1 and Rpb2, form a cleft that contains the active site, identified in the structure by the location of a catalytic magnesium ion. The position of the active site relative to the projected pathway of DNA in the cleft suggests that DNA does not follow a straight path through the enzyme, and two candidate grooves have been identified as exits for nascent RNA. The floor of the Rpb1-Rpb2 cleft contains two pores formed by extensions of Rpb1 and Rpb2 that cross the gap between the two subunits. The pores are at the apex of a funnel-like space in the enzyme. Together, the pores and funnel may function as channels for nucleotides or as channels for RNA and factors that affect 3′-5′ cleavage of nascent RNA during proofreading or bypass of blocks to transcription.

While RNA polymerase II is often considered a single functional unit, its subunits have diverse functions, and there are multiple forms of the core enzyme in living cells. Subunit-specific functions that have been described include start site selection (240, 540), transcriptional elongation rates (12, 222, 455), and interactions with activators (390). The structure of the eukaryotic polymerase also indicates potential functions for particular subunits (122a). Mobile jaws comprised of Rpb5 on one side and Rpb1 and Rpb9 on the other may help position downstream DNA while regions of Rpb1 and Rpb2 together with Rpb6 appear to form a sliding clamp that binds DNA, stabilizing the transcription complex. As expected, Rpb3 and Rpb11 form a structure similar to the $\alpha 2$ dimer and are part of a subcomplex along with Rpb10 and Rpb12.

The yeast Rpb4 and Rpb7 subunits form a dissociable subcomplex that has been implicated in the stress response and in the initiation of transcription (103, 144, 619). While Rpb4 and Rpb7 are present only in substoichiometric amounts during the exponential phase of yeast growth, they are found as stoichiometric subunits of core RNA polymerase II under suboptimal conditions such as the stationary phase of cell growth (103). Recent structural data suggest that Rpb4 and Rpb7 are involved in the interaction between DNA and the active site cleft of RNA polymerase (16, 260). Human Rpb4 and Rpb7 can substitute for their yeast counterparts to a limited extent and display similar patterns of tissue-specific expression (276).

**TABLE 4** RNA polymerase II and the general transcription factors

| Factor | Subunit | Yeast gene | Yeast protein | Features |
|---|---|---|---|---|
| RNA | hRPB1 | *RPB1* | 192 kDa | Contains CTD; binds DNA; involved in start site selection; $\beta'$ ortholog |
| Polymerase II | hRPB2 | *RPB2* | 139 kDa | Contains active site; involved in start site selection, elongation rate; $\beta$ ortholog |
| | hRPB3 | *RPB3* | 35 kDa | May function with Rpb11 as ortholog of the $\alpha$ dimer of prokaryotic RNA polymerase |
| | hRPB4 | *RPB4* | 25 kDa | Subcomplex with Rpb7; involved in stress response |
| | hRPB5 | *RPB5* | 25 kDa | Shared with PolI, II, III; target for transcriptional activators |
| | hRPB6 | *RPB6* | 18 kDa | Shared with PolI, II, III; functions assembly and stability |
| | hRPB7 | *RPB7* | 19 kDa | Forms subcomplex with Rpb4 that preferentially binds during stationary phase |
| | hRPB8 | *RPB8* | 17 kDa | Shared with PoII, II, III; has oligonucleotide/oligosaccharide binding domain |
| | hRPB9 | *RPB9* | 14 kDa | Contains zinc ribbon motif that may be involved in elongation: functions in start site selection |
| | hRPB10 | *RPB10* | 8 kDa | Shared with PolI, II, III |
| | hRPB11 | *RPB11* | 14 kDa | May function with Rpb3 as ortholog of the $\alpha$ dimer of prokaryotic RNA polymerase |
| | hRPB12 | *RPB12* | 8 kDa | Shared with PolI, II, III |

| | | | | |
|---|---|---|---|---|
| TFIIID | TBP | *SPT15* | 27kDa | TATA-binding protein |
| | TAFII250 | *TAFII145/130* | 145 kDa | Involved in promoter binding, G1/S progression; histone acetyltransferase; kinase (human) |
| | CIF150 | *TSM1* | 150 kDa | Involved in promoter binding; mutations arrest in G2/M of cell cycle (yeast) |
| | TAFII130/135 | — | — | Involved in interaction with activators |
| | TAFII100 | *TAFII90* | 90 kDa | Mutations can cause arrest in G2/M of cell cycle (yeast) |
| | TAFII70/80 | *TAFII60* | 60 kDa | Similar to histone H4; binds downstream promoter elements (DPEs) (Drosophila) |
| | TAFII31/32 | *TAFII17* | 17 kDa | Similar to histone H3; interacts with p53 |
| | TAFII20 | *TAFII68/61* | 68 kDa | Similar to histone H2A |
| | TAFII15 | — | — | Similar to histone H2A; highly similar to TAFII20 |
| | TAFII28 | *TAFII40* | 40 kDa | Similar to histone H3; contains atypical histone fold motif seen in Spt3-like transcription factors |
| | TAFII68 | — | — | Contains consensus RNA binding domain; can bind RNA and ssDNA; |
| | TAFII55 | *TAFII67* | 67 kDa | Interacts with numerous activators |
| | TAFII30 | *TAFII23/25* | 23 kDa | Mutations can cause arrest in G1/S of cell cycle (human) |
| | — | *TAFII47* | 47 kDa | No homologous subunit identified in metazoans |
| | — | *TAFII30* | 30 kDA | Shared with TFIIF (yeast), no homologous subunit identified in metazoans |
| | TAFII18 | *TAFII19* | 19 kDa | Similar to histone H4; contains atypical histone fold motif seen in Spt3-like transcription factors |
| | TAFII105 | — | 105 kDa | B-cell specific; related to TAFII130; co-activator for NF-kappaB |

(*Continued*)

**TABLE 4** (*Continued*)

| Factor | Subunit | Yeast gene | Yeast protein | Features |
|---|---|---|---|---|
| TFIIA | TFIIA$\alpha$ | *TOA1* | 32 kDa | Involved in transcriptional coactivation; involved in stabilizing TBP-DNA interactions |
| | TFIIA$\beta$ | — | — | TFIIA$\alpha$ and TFIIA$\beta$ result from processing of a single peptide that is homologous to Toal. |
| | TFIIA$\gamma$ | *TOA2* | 13 kDa | Involved in activator interactions, TFIIA-mediated antirepression, stabilizing TBP-DNA interactions |
| TFIIB | TFIIB | *SUA7* | 38 kDa | Involved in start site selection, promoter binding and promoter bending during initiation |
| TFIIF | RAP74 | *SSU1/TFG1* | 82 kDa | Makes extensive contacts with DNA to position the template during initiation |
| | RAP30 | *TFG2* | 47 kDa | Binds RNA polymerase II and suppresses non-specific DNA binding |
| | — | *ANC1/TFG3* | 27 kDa | Common component of yeast TFIID, TFIIF, and Swi/Snf; similar to ENL and AF-9 proteins |
| TFIIE | TFIIEa | *TFA1* | 55 kDa | Interacts with TFIIH; involved in recruitment, stimulation of TFIIH and promoter opening |
| | TFIIEb | *TFA2* | 37 kDa | Double strand DNA binding activity |

| | | | | |
|---|---|---|---|---|
| TFIIH | p62 | *TFB1* | 73 kDa | Required for nucleotide excision repair; target for activators |
| | p52 | *TFB2* | 59 kDa | Required for nucleotide excision repair |
| | MAT1 | *TFB3* | 32 kDa | Required for nucleotide excision repair; MAT1/Cdk7/cyclin H form the CAK subcomplex (human) |
| | p34 | *TFB4* | 37 kDa | Required for nucleotide excision repair |
| | XPD/ERCC2 | *RAD3* | 90 kDa | 5′–3′ DNA helicase; ATPase; required for DNA repair |
| | p44 | *SSL1* | 52 kDa | Required for nucleotide excision repair; involved in DNA binding and stimulation of XPD activity |
| | XPB/ERCC3 | *SSL2/RAD25* | 95 kDa | 3′–5′ DNA helicase; ATPase; essential for promoter opening and promoter escape |
| | Cdk7 | *KIN28* | 35 kDa | Kinase subunit of cyclin-dependent CTD kinase; Kin28 & cell form the TFIIK subcomplex (yeast) |
| | CyclinH | *CCL1* | 45 kDa | Cyclin subunit of cyclin-dependent CTD kinase |

Since yeast have at least two forms of RNA polymerase II core enzyme (one with Rpb4/7 and one without), and the amounts of these forms depend on the growth and environment of cells (103), it will be interesting to determine whether higher eukaryotes also have multiple forms of core RNA polymerase II, and if these are regulated by development, cell type specificity, or environment.

***RNA Polymerase II CTD*** The largest subunit of RNA polymerase II contains a unique carboxy-terminal repeat domain (CTD) that consists of tandem repeats of a consensus heptapeptide sequence (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) (115, 646). The CTD consensus sequence is highly conserved in eukaryotes, although the number of heptapeptide repeats varies from 26 or 27 in *S. cerevisiae* to 52 in humans. The CTD is essential for viability in yeast and metazoan cells (5, 30, 182, 411, 652).

The functions of the CTD are closely associated with the phosphorylation state of the domain. The CTD exists in at least two phosphorylation states. RNA polymerase II molecules lacking phosphate on the CTD are found in initiation complexes (96, 270, 325, 355, 574), while elongating polymerase molecules contain heavily phosphorylated CTDs (28, 72, 414, 604; reviewed in 127). Since the phosphorylated CTD has a role in recruiting the mRNA capping enzyme to the nascent transcript, and mRNA capping occurs soon after promoter clearance (98, 379, 380, 648), CTD phosphorylation most likely occurs during the transition from transcription initiation to elongation. The switch in CTD phosphorylation states that occurs between initiation and elongation appears to cause the RNA polymerase II molecule to switch cofactors. The Srb/Mediator complex is tightly associated with RNA polymerase II molecules that lack phosphate on their CTDs (289, 301). In contrast, the elongator complex and various RNA processing factors become associated with RNA polymerase II molecules with hyperphosphorylated CTDs (380, 430, 612).

Two kinases have been identified that can phosphorylate the CTD and, based partly on their tight association with the initiation apparatus, are almost certainly involved in regulation of transcription initiation. The Cdk7 subunit of the general transcription factor TFIIH is a CTD kinase (153, 356, 507) and phosphorylation by Cdk7 is thought to be critical for the switch to a stable elongation complex (140, 641). Mutations in the kinase subunit of human TFIIH affect CTD phosphorylation in vitro (562) and in vivo (366). Conditional loss-of-function mutations in the yeast homologue of Cdk7, Kin28, cause a complete loss of transcription of protein-coding genes (236). The Srb10/Cdk8 kinase, a component of the Srb/Mediator complex, is also a CTD kinase (254, 345, 539). Genome-wide expression analysis indicates that yeast Srb10 acts as a negative regulator of transcription under rich growth conditions (236) and a model for its negative regulatory mechanism has been proposed (225).

The proposed role for CTD phosphorylation in transcription elongation is reinforced by evidence that the HIV-1 transcriptional activator Tat enhances transcription elongation by interacting with two CTD kinases, TFIIH and P-TEFb (positive transcription elongation factor b), to stimulate CTD phosphorylation (reviewed in

171, 259, 272, 458, 483, 552, 638). P-TEFb, first identified as a *Drosophila* factor that could stimulate transcription elongation in vitro (373), contains the Cdk/cyclin pair Cdk9 and cyclin T (449, 664). Human cyclin T family members interact with the Tat activator, suggesting that Tat-mediated stimulation of transcription involves recruitment of P-TEFb (605). Human TFIIH has also been reported to bind the activation domain of Tat, although the reports differ on which subunits make contact (125, 172, 437). Tat stimulates the CTD phosphorylation activity of TFIIH. Both TFIIH and P-TEFb CTD kinases seem to be required for Tat-dependent activation in vitro (125, 172, 370, 437, 664).

Fcp1 is a CTD phosphatase with a general role in regulating transcription of protein-coding genes (297). Both yeast and human Fcp1 bind the largest subunit of the general transcription factor TFIIF (11, 13) and both phosphatases are stimulated by the addition of partially purified TFIIF (82, 83). This stimulation can be blocked by addition of TFIIB (84), suggesting that Fcp1, TFIIF, and TFIIB may all regulate polymerase recycling (84, 99). Genome-wide expression analysis with yeast Fcp1 mutants demonstrates that loss of Fcp1 has widespread transcriptional effects (297).

## Basal/General Transcription Factors (Core Promoter Factors)

The set of basal or General Transcription Factors (GTFs) required for specific promoter binding by RNA polymerase II in vitro includes TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (Table 4) (reviewed in 113, 211, 424, 473, 480). Despite the name, it is not yet clear that each of the GTFs functions at all genes in vivo, and it is likely that other components of the transcription apparatus are as generally employed as the GTFs. Hence, these proteins and complexes are best described as core promoter factors, as the general feature of GTFs is that they have roles at core promoters.

A preinitiation complex containing the GTFs and RNA polymerase II can be assembled in a stepwise fashion on promoters in vitro (reviewed in 62, 651). Order of addition experiments indicated that promoter elements were bound by TFIID or TBP, followed by TFIIA, TFIIB, a subcomplex of RNA polymerase II and TFIIF, TFIIE, and finally TFIIH. It now seems unlikely that the individual factors assemble in this fashion at promoters in vivo, for reasons discussed below, but these studies provided a critical framework for understanding the individual roles of the GTFs.

In considering the roles of GTFs, it is useful to keep in mind the multistep models of transcription initiation derived from in vitro studies. The assembled transcription apparatus proceeds through several steps on the way to forming productively elongating complexes (reviewed in 111, 191, 573). The complex melts promoter DNA to form the open complex in which 12–15 bp of promoter DNA are in the form of a single-stranded bubble (open complex formation). Initiation continues with the formation of the first few phosphodiester bonds. Typically, polymerases repeatedly initiate transcription and release the resulting small RNAs

(abortive initiation). Eventually, the polymerase transitions from abortive initiation as it generates a longer RNA (promoter clearance). Polymerase tends to pause 25–30 bp from the start site at many promoters (reviewed in 347, 573). These early elongation complexes need to make a critical transition to a fully competent elongating form of the apparatus that is able to escape the promoter (promoter escape) (reviewed in 111).

We briefly review recent developments with the general transcription factors (GTFs) and refer the reader to other reviews for additional details (113, 211, 424, 473, 480).

***Promoter Binding Factors: TBP, TAFs, TICs*** The structure of TBP, alone and complexed with promoter DNA and GTFs, has been solved (67). TBP resembles a symmetrical molecular saddle (89, 410). When bound to a promoter, this saddle structure sits atop the DNA, contacting the minor groove of the TATA element and inducing a sharp DNA bend accompanied by a partial unwinding of base pairs that may be instrumental in the process of initiation (281, 288).

Additional polypeptides in the TFIID fraction copurify with TBP and were subsequently named TBP-associated factors (TAFs). TAFs were originally described as coactivators (reviewed in, 68, 584), but they are also involved in promoter selectivity (reviewed in 68, 188, 195, 328, 584). TAF function in promoter selection is most clearly illustrated when considering promoters with combinations of TATA and Inr elements. TBP alone is sufficient to direct transcription in vitro from templates with TATA elements. In contrast, TAFs are required for transcription from TATA-less promoters in vitro (65, 375, 378). Basal transcription from TATA-less, Inr-containing promoters requires additional factors (TAF and initiator-dependent cofactors or TICs) that are needed for TAF-dependent Inr functions but are dispensable at TATA-containing promoters (376). TAFs and a subset of TICs are required for the synergistic function of TATA and Inr elements at promoters containing both elements (66, 274, 375, 376, 583). Further evidence for core-promoter selective effects is demonstrated by the observation that TAFs can both inhibit and enhance TBP function, depending largely on the context of the core promoter elements (146, 205, 403, 417, 583). Genetic evidence in yeast and metazoan systems also indicate that TAFs perform promoter selective roles (9, 10, 216, 236, 509, 541, 599).

***TFIIA*** TFIIA functions in part by binding to TBP and stabilizing the TBP-DNA interaction (63, 247). TFIIA interacts with numerous activators (434, 642), and TBP mutants that fail to interact with TFIIA are deficient in activation, suggesting that TFIIA is critical for transcriptional activation of some genes (432, 527). TFIIA may also function by antagonizing transcriptional repressors; it physically displaces or blocks several negative transcriptional regulators from the TFIID complex (17, 177, 252, 382, 385, 433). This mechanism could explain the variable requirement for TFIIA in vitro. TFIIA was originally purified based on its requirement for transcription in crude systems (472). In highly purified systems,

presumably devoid of regulators normally antagonized by TFIIA, TFIIA is dispensable (119, 131, 216, 434, 495, 538, 642).

***TFIIB*** TFIIB is involved in the selection of transcription start sites, possibly by setting distances between promoters and transcription start sites. Mutations in TFIIB cause a shift in the transcription start site (40, 452) and a loss of interaction between TFIIB and RNA polymerase II (26, 40, 64, 70). Structural studies suggest that the distance between TFIIB and the RNA polymerase II catalytic site is approximately 32 bp, which is the average distance between the TATA box element of promoters and transcription start sites (335).

TFIIB interacts with diverse activators that may serve to recruit TFIIB to promoters (285, 346). The strength of certain activator-TFIIB interactions correlates with the potency of the activator (346, 622), although analysis of various TFIIB point mutants defective in activation in vitro has not perfectly mirrored the behavior of these mutants in vivo (104). Studies with activation-defective TFIIB mutants suggest that TFIIB-binding activators may function in some cases by inducing conformational changes in TFIIB (2, 621).

***TFIIF*** TFIIF has many of the characteristics of bacterial sigma factor: It binds tightly to RNA polymerase II, suppresses nonspecific DNA binding, and stabilizes the preinitiation complex (112, 196). Like sigma factor, TFIIF can bind *Escherichia coli* polymerase, and this binding occurs through regions of the Rap30 subunit that are most similar to sigma factor (381).

TFIIF may also function by affecting DNA topology. Crosslinking experiments with purified transcription factors suggest that DNA is wrapped one complete turn around the preinitiation complex (477). TFIIF is critical for tight wrapping of DNA, possibly inducing torsional strain in the DNA, thereby facilitating promoter melting. Mutants in TFIIF that display transcription defects are also defective in DNA wrapping (122, 477).

TFIIF can also stimulate polymerase elongation rates by suppressing transient pauses during transcription (35, 161, 257, 460). It is unknown whether this function is related to its DNA wrapping ability, association with known elongation cofactors (280), or a recently discovered intrinsic kinase activity (484).

***TFIIE*** TFIIE function is closely linked to TFIIH: Interspecies complementation of TFIIE function in vitro is possible only if both TFIIE and TFIIH are from the same organism (343). In stepwise assembly models, TFIIE follows RNA polymerase II, precedes TFIIH, and possibly functions in TFIIH recruitment (63, 160). TFIIE also stimulates the CTD kinase and ATPase activities of TFIIH (356, 421–423).

TFIIE is likely to play a role in melting of promoter DNA. Both human and yeast TFIIE contain a zinc ribbon motif that is implicated in DNA binding (463) and TFIIE can bind regions of single stranded DNA (311), suggestive of a role in opening or maintaining an open promoter complex. Significantly, the in vitro

requirement for TFIIE can be bypassed by premelted promoter sequences (237, 436). This requirement for TFIIE is influenced by helical stability of the DNA substrate (237). As stability is a function of sequence, one prediction is that the requirement for TFIIE should be sequence and therefore promoter specific. Indeed, genes show variable requirements for TFIIE both in vivo and in vitro (236, 237, 444, 491, 560).

***TFIIH*** TFIIH can be separated into two subcomplexes, core TFIIH and a separable kinase/cyclin subcomplex (154, 542, 544, 600, 603). Core TFIIH is also found as a subcomplex of the nucleotide excision repair complex (134, 241, 497, 498, 603), which accounts for previous observations of coordinated transcription and DNA repair (46, 384). The subunits of TFIIH have at least three enzymatic activities: DNA-dependent ATPase, ATP-dependent helicase, and CTD kinase (reviewed in 107).

TFIIH performs critical roles in multiple, early steps of transcription. Two subunits have helicase activity (XPB/ERCC3 and XPD/ERCC2 in human, Rad25/Ssl2 and Rad3 in yeast). The helicase activity found in XPB is essential for promoter opening in vitro (208, 290). Requirements for this function can be bypassed by use of supercoiled (442, 443, 572) or premelted templates (237, 436, 550), further supporting a role for TFIIH in promoter opening. This helicase activity is also required for the transition from abortive to productive elongation. XPB prevents premature arrest of early elongation complexes at promoter-proximal positions (51, 140, 141, 235, 238, 312, 429).

The TFIIH kinase/cyclin pairs are Cdk7/cyclin H in humans and Kin28/Ccl1 in yeast (487, 508, 512). These Cdks phosphorylate serines in the RNA polymerase II CTD. TFIIH can phosphorylate additional substrates, although it is unclear whether all of these are physiologically relevant targets (422, 478). In mammalian systems, the TFIIH kinase also functions as a Cdk activating kinase (CAK) and regulates cell cycle transitions (155, 156, 367, 454, 487, 508, 512, 523). In yeast, the corresponding CAK activity is performed by a distinct kinase (Cak1/Civ1) and not by the yeast TFIIH kinase (Kin28) (150, 268, 559). Cak1 can phosphorylate Kin28, and phosphorylation of TFIIH may serve to link transcription and the cell cycle (290).

Mutations in the XPB and XPD helicases are responsible for several genetic diseases in humans including xeroderma pigmentosum, Cockayne's syndrome, and trichothiodystrophy (reviewed in 107). While XPB appears to be the primary helicase involved in transcription, XPD is required for nucleotide excision repair (610). The complex phenotypes of these TFIIH-related diseases will likely relate to both transcriptional and repair defects (107).

## Yeast Polymerase II Holoenzyme

The yeast RNA polymerase II holoenzyme is a complex of RNA polymerase II, a subset of the general transcription factors, and the Srb/Mediator complex

(289, 301). The holoenzyme was discovered when attempts to purify Srb proteins using conventional column chromatography led to the identification of a large complex that also contained RNA polymerase II and GTFs (301, 556). The purified RNA polymerase II holoenzyme has the capacity to initiate transcription and respond to activators when supplemented with additional purified general transcription factors (301). In contrast, transcription reactions containing highly purified yeast core RNA polymerase II and GTFs are unresponsive to activators (157, 494, 495). The ability of Srb-containing holoenzymes to respond to activators in vitro reflects the apparent functions of *SRB* (Suppressor of RNA polymerase B) gene products in vivo. *SRB*s had been identified in a genetic selection for factors involved in RNA polymerase II CTD function (412); the CTD mutations used in the selection caused defects in transcription activation in vivo (496) and in extracts in vitro (344).

A second line of investigation proved critical in the discovery of the yeast holoenzyme and elucidation of its functions (289). The holoenzyme can be separated into core RNA polymerase II, general transcription factors, and a CTD-associated subcomplex by incubating holoenzyme preparations with anti-CTD antibodies. The subcomplex dissociated from the CTD sufficed to reconstitute the response to an activator when added to purified core RNA polymerase II and GTFs; thus, the complex was named the mediator of activation. The mediator contains the Srb proteins and other holoenzyme-associated regulatory proteins (226, 289).

There are approximately 2000 to 4000 molecules of RNA polymerase II holoenzyme in haploid yeast cells (302). This contrasts with 10,000 to 20,000 molecules of RNA polymerase II, similar levels of GTFs, and approximately 2,000,000 ribosomes per cell (328). The Srb proteins may be limiting for holoenzyme formation, since all Srb protein in yeast cells is associated with RNA polymerase II when cells are harvested (302).

The Srb-containing RNA polymerase II holoenzyme is essential for transcription of most protein-coding genes in yeast cells. Analysis of temperature-sensitive mutants of two of the Srb components, Srb4 and Srb6, demonstrated that they were globally required for transcription in yeast cells (557), and subsequent genome-wide expression analysis confirmed these results (236). Since the yeast Srb proteins are found tightly and exclusively associated with RNA polymerase II when cells are harvested, an Srb-containing RNA polymerase II holoenzyme is probably required at most promoters in vivo.

***Heterogeneity in Holoenzyme Composition*** The Srb-containing RNA polymerase II holoenzymes purified thus far are remarkably similar, despite differences in yeast strains, cell growth conditions, and purification protocols (226, 289, 300, 301). The differences noted in subunit composition involve the set of GTFs and the number of regulatory complexes that copurify. Some yeast holoenzyme preparations contain all of the GTFs except TBP and TFIIE (301); some contain all of the GTFs except TBP, TFIIB, and TFIIE (300); and others have been isolated with only a single GTF (TFIIF) (289). Some yeast holoenzyme preparations contain stoichiometric levels of Swi/Snf (609), whereas others lack any detectable

Swi/Snf protein (74, 289). It seems likely that RNA polymerase II holoenzymes in living cells exist in more than one form and this may be partially responsible for the differences in purified holoenzymes. Also, large multisubunit complexes do not easily survive exposure to the charged resins used in conventional column chromatography. The ribosome, for example, dissociates into subcomplexes when subjected to ion exchange chromatography (132). For this reason, the exact composition of RNA polymerase II holoenzymes in vivo is not known. The holoenzyme discussed here is composed of core RNA polymerase II, all the GTFs other than TBP (and its associated proteins), the core Srb/Mediator complex, and the Srb10 cyclin-dependent kinase complex.

The composition of the Srb/Mediator complex can be remodeled as cells encounter new environments to allow coordinate control of specific sets of genes (236). The Srb10 Cdk is a component of the Srb/Mediator complex of RNA polymerase II holoenzymes in cells growing exponentially on rich glucose media, but becomes depleted from cells as they enter the diauxic shift, where glucose becomes limiting. Since Srb10 is a repressor of glucose-repressed genes (among others), this depletion provides a mechanism to coordinately induce the population of glucose-repressed genes. It will be important to determine whether cells remodel the Srb/Mediator complex in additional ways. Many components of the Srb/Mediator complex have critical roles at only a subset of genes, and coordinate regulation of such genes could occur through regulation of the composition or modification of Srb/Mediator complexes (236).

## Yeast Srb/Mediator Complex and Subcomplexes

***Srb/Mediator Function*** Many components of the Srb/Mediator complex play essential roles in transcriptional regulation in living cells, apparently by providing targets for transcriptional activators and conveying regulatory signals to RNA polymerase II and other components of the initiation apparatus. Genetic studies suggest that Srb function is associated with the CTD, and CTD-proximal regions of the large subunit of RNA polymerase II (412) and purified Srb/Mediator complex binds tightly and specifically to recombinant CTD (400). The Srb/Mediator complex can stimulate CTD phosphorylation by TFIIH in vitro (289), possibly providing the switch between initiation and elongation forms of the polymerase. Analysis of DNA-bound and free forms of RNA polymerase II in crude cell extracts suggests that the Srb/Mediator complex is not associated with elongating RNA polymerase II, but rather is limited to the initiating form of RNA polymerase II (543).

***Srb/Mediator Composition*** The Srb/Mediator complex (Figure 5) can be purified by treating holoenzyme preparations with anti-CTD antibodies (226, 289), by affinity purification with recombinant CTD columns (464), and by conventional column chromatography (400). The most highly purified complex, considered the "core" mediator complex, is composed of around 20 polypeptides: Srb2, Srb4,

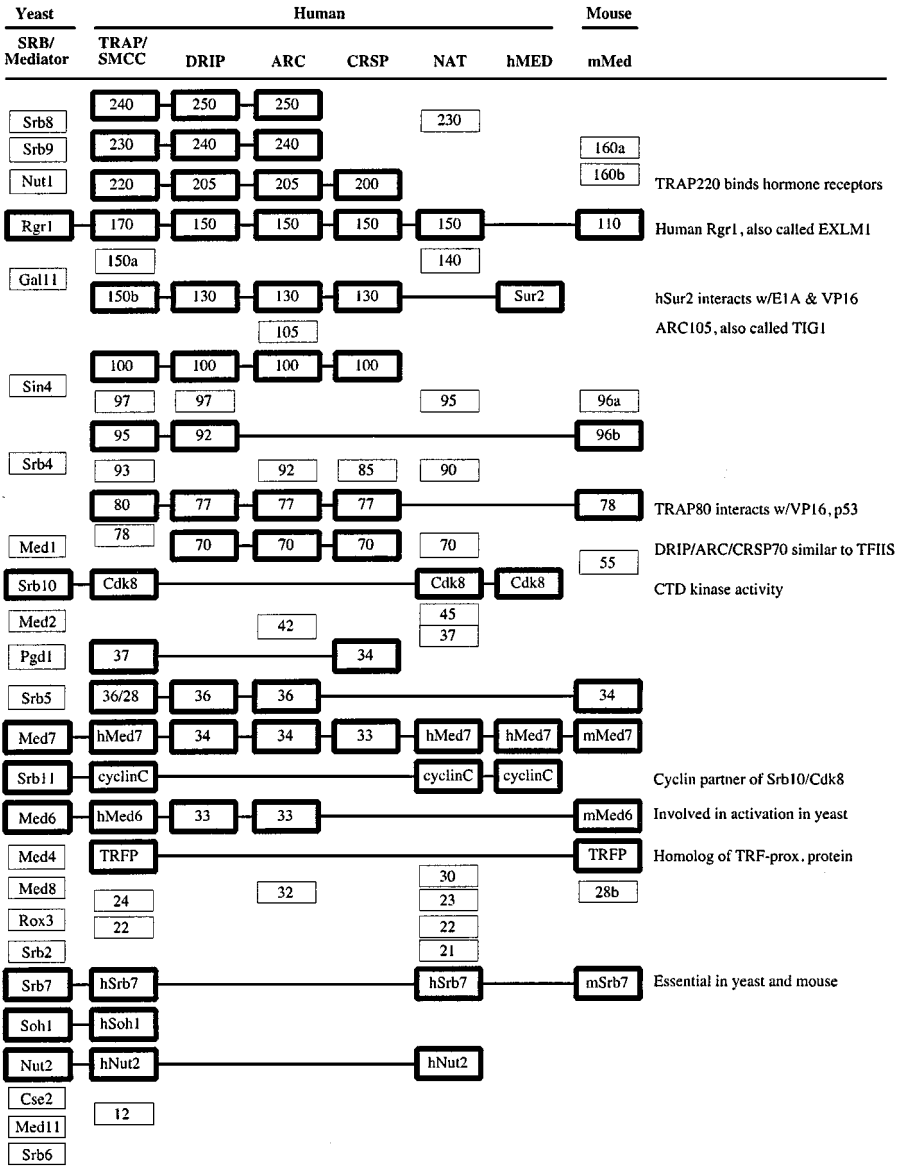


**Figure 5** Subunit composition of Srb/Mediator-like complexes. The subunits of yeast, human, and mouse SRB/mediator-like complexes are compared. Identical or orthologous subunits are marked by bold boxes and connected by lines. For metazoan subunits that have not been named, numbers indicate subunit size in kDa. Soh1 has not yet been confirmed as a component of the yeast Srb/Mediator. Additional information can be found at http://web.wi.mit.edu/young/pub/srbmediator.html

Srb5, Srb6, Srb7, Med1, Med2, Med4, Med6, Med7, Med8, Med11, Cse2, Gal11, Pgd1, Rgr1, Rox3, Sin4, and Nut2 (400). Srb/Mediator complexes derived from RNA polymerase II holoenzymes obtained from exponentially growing cells also contain an Srb10 Cdk complex (Srb8, Srb9, Srb10, Srb11) (226, 345).

On the basis of their genetic and biochemical properties, the components of the Srb/Mediator complex can be classified into multiple categories, as described below.

***Srb2, Srb4, Srb5, Srb6, Med6, and Rox3 Subcomplex*** There is considerable evidence for a Srb2, Srb4, Srb5, Srb6, Med6, Rox3 subcomplex that has roles in holoenzyme stabilization and transcription activation. The *SRB2*, *SRB4*, *SRB5*, and *SRB6* genes were originally identified as dominant suppressors of RNA polymerase II CTD truncation mutants (299, 412, 556). Although CTD truncation does not seem to alter core RNA polymerase II stability (411), it reduces the ability of the holoenzyme to respond to activators (4, 344, 496). The *SRB* gain-of-function mutations compensate for CTD truncation by affecting the ability of activators to interact with the holoenzyme, as with *SRB4-1* (298), or by stabilizing the holoenzyme, as with *SRB2-1* (152). The holoenzyme-stabilizing role of Srb2 is also consistent with in vitro assays showing that Srb2 and Srb5 are required for transcription and assembly of a stable PIC in nuclear extracts (299, 556). Mutations in genes encoding Srb2 and Srb5 produce nearly identical phenotypes, suggesting that Srb2 and Srb5 have similar roles in holoenzyme stabilization (299, 556).

Biochemical and genetic assays demonstrate that Srb4 is a direct target of the Gal4 activator (298). This role for Srb4 in activation may be shared by Srb6 as well. Both proteins are encoded by essential genes that affect transcription of virtually all protein-coding genes in vivo (236, 556, 557). A temperature-sensitive *srb4* mutant that produces a rapid and general shutdown of mRNA synthesis (236, 557) can be suppressed by a dominant (gain-of-function) mutation in *SRB6* or *MED6* (327). Conversely, a *med6-ts* mutation can be suppressed by a dominant allele of *SRB4* (329). In this case, suppression of the *med6-ts* growth defect correlates with a partial rescue of the transcriptional defects in vivo (329). These genetic suppression interactions are allele-specific, suggesting that direct physical contacts link Srb4 to Srb6 and Med6.

Med6 contributes to the activation function of the Srb/Mediator subcomplex, as it is required for the full induction of a subset of genes in vivo and in vitro (330). Genome-wide expression analysis reveals that approximately 10% of yeast genes depend on Med6 function (236). In vitro transcription experiments with RNA polymerase II holoenzymes impaired for Med6 function showed that Med6 is required for activated transcription by the VP16 acidic activator (330).

Consistent with the genetic evidence implicating these proteins in similar functional pathways, recombinant Srb2, Srb4, Srb5, Srb6, and Med6 can form a stable complex in which Srb2 associates with Srb5, and Srb4 associates with Srb6 and Med6 (298, 327). A bridging interaction between Srb2 and Srb4 brings the

complete set of proteins together in a single complex. A purified yeast Srb/Mediator subcomplex can be disrupted into two small stable subcomplexes after denaturing urea treatment (329). One subcomplex contains Srb2, Srb4, Srb5, Srb6, Med6, and Rox3. The other is reported to contain Srb7, Med1, Med4, Med7, Med8, Med9, Gal11, Pgd1, Rgr1, and Sin4.

***Gal11, Pgd1 (Hrs1/Med3), Rgr1, Sin4, and Med2*** Gal11, Pgd1, Rgr1, Sin4, and Med2 may form a subcomplex or functional module within the Srb/Mediator complex, and several of these proteins have been implicated in transcription activation. A direct physical interaction between Gal11, Pgd1, Rgr1, Sin4, and Med2 has been inferred from the biochemical analysis of RNA polymerase II holoenzymes purified from yeast strains deleted for *MED2*, *PGD1*, and *SIN4* or partially deleted for *RGR1* (341, 399). The pattern of subunits missing from the holoenzyme preparations predicts a Med2/Pgd1 subcomplex, Gal11 bound to Sin4, and Sin4 in turn anchored to RNA polymerase II holoenzyme by Rgr1. To explore the functions of these proteins, the mutant holoenzymes were assayed for basal transcription, activated transcription by VP16 and Gcn4, and TFIIH CTD phosphorylation. Stimulation of basal transcription and TFIIH-dependent CTD phosphorylation was relatively unaffected by loss of Med2, Pgd1, Sin4, or Rgr1, whereas activated transcription by VP16 required the Med2/Pgd1 subcomplex, and Gcn4-dependent activation required Sin4 (399). Similarities among mutant phenotypes and genetic suppression analysis also suggest a functional link between Gal11, Pgd1, Rgr1, and Sin4 (79, 341, 453).

***Srb8, Srb9, Srb10, and Srb11 Subcomplex*** A subcomplex of Srb8, Srb9, Srb10, and Srb11 is involved in repression of specific sets of genes, and is itself regulated in response to the environment of the cell. Srb8, Srb9, Srb10, and Srb11 copurify with RNA polymerase II holoenzymes and Srb/Mediator complexes isolated from cells growing exponentially in rich glucose medium (226, 345; D Chao, V Myer & R Young, unpublished). Srb10 is a cyclin-dependent kinase and Srb11 is its cyclin partner (345). Several lines of genetic and biochemical evidence indicate that Srb8, Srb9, Srb10, and Srb11 are all functionally and physically associated. RNA polymerase II holoenzyme purified from a *srb10*$\Delta$ strain lacks Srb8, and holoenzyme from a *srb8*$\Delta$ strain lacks Srb10 and Srb11 (398). Loss-of-function mutations in the *SRB8*, *SRB9*, *SRB10*, and *SRB11* genes all suppress CTD truncation mutations and produce cells that are phenotypically indistinguishable (226, 345). The four Srb proteins coelute identically from columns during holoenzyme purification. Recombinant Srb10 and Srb11 can form a functional complex capable of CTD phosphorylation in vitro (225).

Analysis of Srb8, Srb9, Srb10, and Srb11 function in vivo and in vitro indicates that they are involved in regulating a small but important set of genes, and that their function is predominantly a negative one. Loss-of-function mutations in the *SRB8*, *SRB9*, *SRB10*, and *SRB11* genes partially restore the defects due to CTD truncation mutations (226, 345). Many genetic screens and selections have

found that *SRB8, SRB9, SRB10,* and *SRB11* are required for complete repression of transcription of a variety of genes, consistent with the role of their protein products in inhibition of transcription (23, 114, 226, 310, 345, 524, 545, 595; reviewed in 79).

Genome-wide expression analysis with mutant cells revealed that the Srb10 kinase is a negative regulator of a substantial fraction of genes that are repressed when cells grow vegetatively in rich media and are induced as cells experience nutrient deprivation (236). The genes regulated by Srb10 include glucose-repressed genes and genes that are critical for the morphological change that permits foraging for nutrients and stress responses. Srb10 is physically depleted from cells as they enter the diauxic shift, providing a mechanism for derepression of these sets of genes. Srb10 in wild-type cells is thus responsible for repressing these genes when cells are in exponential growth on glucose, but no longer performs this function as cells enter the diauxic shift.

Srb10 and Srb11 mRNA and protein levels do not vary substantially with the cell cycle (114; J Zhang & R Young, unpublished). Srb11 protein levels decrease at the onset of meiosis and after heat shock (114). This suggests that cells remodel holoenzyme composition or function with respect to the Srb10 kinase complex during stationary phase, meiosis, and heat shock.

Experiments with wild-type and mutant holoenzymes indicate that Srb10 is uniquely capable of phosphorylating the CTD of holoenzyme molecules prior to stable preinitiation complex formation, thereby inactivating the holoenzyme for transcription initiation (225). Negative regulation of some promoters may therefore occur by stimulating an otherwise repressed Srb10 kinase prior to stable initiation complex formation.

There is also evidence consistent with a positive role for Srb10 at one set of genes and under certain growth conditions. Cells that lack Srb10 function are not fully capable of inducting Gal gene expression (345). Srb10 can phosphorylate the Gal4 transcriptional activator, and this may potentiate the ability of Gal4 to activate transcription (232).

***Other Subunits of the Srb/Mediator Complex Srb7*** The gene for Srb7 was isolated as a recessive suppressor of CTD truncation, and is essential for cell viability (226). Srb7 is tightly associated with the core Srb/Mediator complex, unlike proteins encoded by the other *SRB* genes that were isolated as recessive suppressors (*SRB8, SRB9, SRB10,* and *SRB11*) (400). Genome-wide expression analysis of Srb7 mutants indicates that expression of at least half the genome is dependent on Srb7 function (S Hassan, H Causton & R Young, unpublished).

***Cse2, Nut1, and Nut2*** Cse2, Nut1, and Nut2 are small holoenzyme subunits whose genes were previously identified in unrelated genetic screens (206). *CSE2* was originally isolated as a consequence of its effects on chromosome segregation (626). *NUT1* and *NUT2* were isolated in a selection for factors that, when mutated, relieved the requirement for Swi4-mediated regulation at URS2 of the *HO* gene

(545). This same screen also identified genes encoding many other Srb/Mediator subunits (*SIN4, ROX3, SRB8, SRB9, SRB10, and SRB11*).

***Med proteins (Med1, Med4, Med7, Med8, and Med11)*** The Med proteins were identified during biochemical fractionation as components of the Srb/Mediator complex that, at the time, could not be related to a previously identified gene (206, 400). Med3, Med9, and Med10 have since been identified as the products of *PGD1* (*HRS1*), *CSE2*, and *NUT2*, respectively. Med1 mutations have both positive and negative effects on transcription, and produce phenotypes similar to those of Srb10 mutations. The protein product probably interacts with Med2 (22). Med4 and Med5 are a single protein migrating as a doublet (400) and the corresponding gene has been named *MED4*. Med8 was recently reported to bind directly to regulatory elements of some genes, suggesting a mechanism to link activation and repression complexes to the transcriptional machinery (91).

***Additional candidates*** Soh1 has been implicated in genetic studies as a factor that interacts with components of the general transcription machinery (151). The mammalian homologue of Soh1 has been identified in mammalian Srb/Mediator-like coactivator complexes (202). The yeast protein Xtc1 can be detected in partially purified RNA polymerase II holoenzyme preparations, and may interact with multiple activators (147).

## Cdc73/Paf1 RNA Polymerase II Complex

An RNA polymerase II complex that contains Cdc73, Paf1, Ccr4, Hpr1, Gal11, Sin4, and Rgr1, but lacks many components of the Srb/Mediator complex, has been described (86, 87, 511, 594). Although this complex has yet to be fully purified and characterized, partial purification of tagged forms of Cdc73 and Paf1 indicates that these proteins associate with a form of RNA polymerase II that lacks components of the Srb/Mediator complex such as Srb2, Srb4, Srb5, and Srb6 (511). The Cdc73- and Paf1-containing complex has been proposed to be a form of RNA polymerase II holoenzyme, distinct from that containing the Srb/Mediator complex, that coexists with the major form of holoenzyme (86, 87). In this model, the major form of holoenzyme (containing the Srb/Mediator complex) initiates transcription of most genes, while the Cdc73/Paf1-containing holoenzyme is involved in transcription of a smaller, overlapping set of genes, which include genes regulated by protein kinase C (86).

The Srb/Mediator-containing RNA polymerase II holoenzyme and its Srb/Mediator complex has been purified and characterized in various in vitro assays, and it will be important to subject the putative Cdc73/Paf1-containing holoenzyme to a similar evaluation to better understand its functions. Furthermore, transcription of at least 95% of yeast genes is dependent on the Srb4-containing RNA polymerase II holoenzyme, including many genes regulated by protein kinase C (236), so it will be interesting to ascertain the relative roles of the two

RNA polymerase II complexes in transcription of genes that are dependent on the Cdc73/Paf1-containing holoenzyme.

## Metazoan Holoenzymes and Coactivators

Metazoan RNA polymerase II holoenzymes exhibit many of the features of the yeast RNA polymerase II holoenzyme, but are less stable to conventional column chromatography than their yeast cousins and appear to be more diverse. Although much of the work in this area is very recent, and a broad range of interesting issues remains, several conclusions have emerged from studies of metazoan RNA polymerase II holoenzymes and their Srb/Med-containing coactivator complexes. First, although it has not been possible to purify to homogeneity intact RNA polymerase II holoenzymes using conventional ion exchange column chromatography, affinity methods have permitted isolation and characterization of these large complexes (101, 254, 427, 428, 435, 539), and Srb-containing holoenzymes have been partially purified using conventional methods (88, 369, 408). Second, a set of coactivator complexes purified from a variety of systems are almost certainly homologues of the yeast Srb/Mediator complex (48, 202, 254, 262, 369a, 401, 466, 489). Third, several important features of the holoenzymes and their Srb/Med-containing coactivators are conserved from yeast to humans, including their role in responding to gene-specific transcriptional activators and repressors.

As mentioned previously, the model emerging from recent studies is that a transcription initiation apparatus that approximates the size and complexity of the ribosome is assembled at promoters under the control of, and with the capacity to respond to, combinations of gene-specific regulators bound to enhancers. The modularity of the transcription initiation apparatus enables the cell to recruit components of this initiation apparatus in multiple steps or, if the apparatus is already fully assembled, in a single concerted step.

***Isolation and Characterization of Metazoan Holoenzymes*** RNA polymerase II holoenzyme complexes from mammalian cells have been partially purified by following the presence of human Srb7 (88, 369), the CTD kinase Cdk7 (185, 427, 428), or the general transcription factor TFIIF (100, 369). Mammalian holoenzymes have also been identified and characterized based on their interactions with transcriptional activators (48, 125, 438) and elongation factors (435). The mammalian RNA polymerase II holoenzymes best characterized for subunit composition have stoichiometric levels of RNA polymerase II and all the GTFs except for TFIID (435).

The Srb proteins have been considered a hallmark of the yeast RNA polymerase II holoenzyme since they are found almost entirely associated with the holoenzyme when yeast cells are harvested (302). Most of the metazoan holoenzyme preparations described thus far contain homologues of the yeast Srb/Med proteins. Among the various Srb/Med proteins, the sequence and function of Srb7 is highly conserved between yeast and humans (88). All of the RNA polymerase II holoenzyme preparations examined for the presence of mammalian Srb7 contain this Srb

protein (88, 369, 408, 427; J Greenblatt, personal communication). In addition, when crude extracts from mammalian cells were subjected to density gradient centrifugation, all of the detectable Srb7 was associated with high-molecular-weight forms of RNA polymerase II (427). The gene encoding Srb7 is expressed in most mouse tissues and is essential for viability (571), suggesting that Srb7-containing polymerase complexes exist in most tissues and play essential roles in expression of protein-coding genes.

Homologues of several additional yeast Srb and Med proteins are found in mammalian holoenzyme complexes. Cdk8 and cyclin C share sequence and functional homology with Srb10 and Srb11, respectively, and are generally components of mammalian holoenzyme preparations (reviewed in 445). Orthologues of yeast Srb7, Srb10, Srb11, Med6, Med7, Nut2, Rgr1, and Soh1 have been identified in mammalian coactivator complexes (202, 262, 369a). Mammals and *Caenorhabditis elegans* have genes that appear to encode homologues of Rgr1 (262), and Nut2 (206, 545).

Additional study of preparations of mammalian RNA polymerase II complexes revealed the presence of various proteins previously identified as transcription or DNA repair factors, including the chromatin-modifying factors BRG1, Swi/Snf, and PCAF (102, 408), the coactivator CBP (408), the tumor suppressor gene product BRCA1 (6, 504), DNA repair proteins (369), and the MCM family of DNA replication factors (640). Further study is needed to ascertain how these physical interactions reflect the functional interactions among these complexes in living cells.

***Metazoan Srb/Mediator-Like Coactivators*** The metazoan Srb/Mediator-like complexes that have been described include TRAP/SMCC (202, 254), ARC (401), DRIP (466), NAT (539), murine mediator (262), CRSP (489), and a human Sur2-containing complex (48).

The best-characterized metazoan Srb/Mediator-like complex is the Srb and Med Cofactor Complex (SMCC), which was isolated from human cell lines with epitope-tagged Srb7, Srb10, or Srb11 on the basis of its ability to mediate activation by Gal4 derivatives (202). SMCC contains approximately 25 proteins, including orthologues of yeast Srb7, Srb10, Srb11, Med6, Med7, Nut2, Rgr1, and Soh1. As with the yeast Srb/Mediator complex, highly purified SMCC does not contain RNA polymerase II. SMCC is essentially identical to another coactivator complex, TRAP (Thyroid Receptor Associated Proteins), that was purified independently for its ability to mediate activation with thyroid hormone receptor (163, 164, 254), so this complex is now called TRAP/SMCC. TRAP/SMCC can bind simultaneously to activators and ligand-bound thyroid hormone receptor, indicating that the complex is capable of integrating multiple signaling inputs (254).

ARC (Activator-Recruited Cofactor) was identified as a cofactor required for synergistic activation by the Sp1 and SREBP-1a activators (401). DRIP (Vitamin-D-Receptor Interacting Protein) was originally identified as a complex of proteins that interact specifically with ligand-bound vitamin D receptor (467). Purification

of both complexes and identification of individual subunits led to the realization that the two complexes were essentially identical. ARC/DRIP can bind a number of diverse activators and ligand-bound nuclear hormone receptors (401, 466). A substantial number of the ARC/DRIP subunits are also found in the CRSP coactivator, which was purified by its requirement for the stimulatory activity of the enhancer-binding protein Sp1 (489).

NAT (Negative Regulator of Activated Transcription) was isolated by affinity purification of the human homologue of yeast Srb10 (539). NAT is capable of repressing activated transcription under certain conditions in vitro, as is SMCC (369a), consistent with the presence of homologues of yeast Srb10 and Srb11, which can function as a repressor in vivo and in vitro (225, 236).

The functions of the metazoan Srb/Mediator-like complexes and the identity of specific subunits of TRAP/SMCC, ARC/DRIP, CRSP, and NAT (Figure 5) indicate that these complexes are highly related. It is possible that each of these complexes is derived from a single metazoan Srb/Mediator complex in vivo, and that differences in biochemical fractionation and assays produce diverse fractured elements of the complex. It is also likely that metazoan Srb/Mediator complexes exist in multiple forms in cells, and can vary in subunit composition to reflect the requirements of specific cells and environments.

***Additional Metazoan Cofactors*** There are additional positive and negative cofactors involved in transcription initiation by RNA polymerase II. The positive cofactors include PC1, PC2, PC3/Dr2, PC4, p52 and p75, PC5, PC6, and PC7. The negative cofactors include NC1 and NC2 (DR1-DRAP1) (reviewed in 267, 481).

## RNA POLYMERASE II AND ELONGATION COFACTORS

### A Switch from Initiation to Elongation

To produce an RNA transcript, the formation of a stable transcription initiation complex must be followed by promoter clearance and processive elongation. Several lines of evidence indicate that the switch from initiation to elongation involves phosphorylation of the RNA polymerase II CTD and an exchange of cofactors associated with the polymerase. RNA polymerase II molecules found in initiation complexes lack phosphate on their CTDs, while elongating polymerase molecules contain heavily phosphorylated CTDs (reviewed in 127). The Srb/Mediator complex is tightly associated with RNA polymerase II molecules that lack phosphate on their CTDs in the holoenzyme (289, 301). In contrast, the elongator complex and various RNA processing factors become associated with RNA polymerase II molecules with hyperphosphorylated CTDs (380, 430, 612). CTD phosphorylation must occur during the transition from transcription initiation to elongation, because the phosphorylated CTD has a role in recruiting the mRNA capping enzyme to the nascent transcript, and mRNA capping occurs soon after promoter

clearance (98, 379, 380, 648). The precise mechanisms that control the switch from initiation to elongation are unknown.

Several factors have been identified that regulate promoter clearance, promoter escape, and processivity of elongation (summarized in Table 5).

## Factors Influencing Promoter Clearance and Escape

Studies of transcription with purified RNA polymerase II suggest that promoter clearance and escape constitutes an important regulatory step. Promoter clearance and escape appears to involve a balance between negative and positive regulation by various factors (reviewed in 347, 360, 448, 475, 513, 515, 573).

***Negative Elongation Factors*** Transcription release factor 2 was originally identified as a factor that suppresses the appearance of long transcripts (459) and effects ATP-dependent release of nascent transcripts (350, 628). Two factors, DSIF and NELF, can confer sensitivity to the transcription inhibitor DRB, which inhibits mRNA synthesis and CTD phosphorylation (137). DSIF was isolated by its ability to induce pausing of the transcription complex in conjunction with the transcription inhibitors DRB and H8 (591). The two subunits of DSIF are homologous to the Spt4 and Spt5 gene products of *S. cerevisiae* and genetic studies link the function of these genes to elongation (217, 591, 632). NELF also confers DRB sensitivity (630) and functions cooperatively with DSIF in repressing transcription.

***Positive Elongation Factors*** P-TEFb, TFIIF, and TFIIH contribute to formation of elongation-competent complexes. The activity of P-TEFb counters the negative activities of NELF and DSIF (reviewed in 631). P-TEFb was originally purified as a factor that stimulates transcriptional elongation and phosphorylates the CTD in *Drosophila* (374, reviewed in 458). Subunit identification revealed that *Drosophila* P-TEFb consists of a kinase/cyclin pair (Cdk9/cyclin T) (168, 449, 450, 605). A human homologue of Cdk9 (PITALRE) was identified by sequence homology (664). Cdk9 was simultaneously found to be a cofactor for stimulation of transcription by HIV Tat protein, thus identifying human P-TEFb as a Tat-associated kinase (TAK) (186, 370, 636, 664). The Tat coactivator of HIV-1 may also stimulate transcription by its ability to recruit the elongation factor Tat-SF1 (663), which may function as a general elongation factor (339).

TFIIF and TFIIH, which clearly have roles in transcription initiation, also affect the elongation stage of transcription (35, 81, 162, 460). TFIIF lowers the frequency of abortive initiation events and helps prevent premature stalling of early elongation complexes (633). TFIIF may leave polymerase just after initiation, but may then be re-recruited by paused polymerase (650). The mechanism of elongation stimulation by TFIIF is unclear, but may be related to TFIIF's ability to promote the wrapping of DNA around RNA polymerase II (477), its association with elongation factors (280), or a recently described kinase activity (484). TFIIH kinase activity may play a role in elongation through CTD phosphorylation, but it appears

**TABLE 5** Eukaryotic elongation factors[a]

| Complex | Function | Subunit | Size |
|---|---|---|---|
| DSIF | Negative factor, renders elongation sensitive to kinase inhibitor DRB | hSpt5 | 160 kDa |
| | | hSpt4 | 14 kDa |
| NELF | Negative factor, functions with DSIF | NELF-A | 66 kDa |
| | | NELF-B | 61 kDa |
| | | NELF-C | 59 kDa |
| | | NELF-D | 58 kDa |
| | | NELF-E/RD | 46 kDa |
| Factor 2 | Negative factor, causes transcript release | Factor 2 | ~110 kDa |
| P-TEFb | Position factor, may function through CTD phosphorylation, Cdk9 can partner with 1 of at least 4 cyclins | Cdk9 | 43 kDa |
| | | Cyclin: T1 | 81 kDa |
| | | T2a | 74 kDa |
| | | T2b | 81 kDa |
| | | K | 41 kDa |
| TFIIH | Positive factor, may funtion through CTD phosphorylation | XPB/ERCC3 | 89 kDa |
| | | XPD/ERCC2 | 80 kDa |
| | | p62 | 62 kDa |
| | | p52 | 52 kDa |
| | | p44 | 44 kDa |
| | | Cdk7 | 40 kDa |
| | | Cyclin H | 34 kDa |
| | | p34 | 34 kDa |
| | | MAT1 | 32 kDa |
| TFIIF | Suppresses transient pausing and premature arrest | RAP74 | 74 kDa |
| | | RAP30 | 30 kDa |
| FACT | Facilitates transcription elongation through nucleosomes | hSpt16 | 140 kDa |
| | | SSRP1 | 80 kDa |
| SII | Relieves transient pausing and arrest | TFIIS | 32 kDa |
| Elongin (SIII) | Increases rate of elongation | ElonginA | 110 kDa |
| | | ElonginB | 18 kDa |
| | | ElonginC | 15 kDa |
| Holo-ELL | Increases rate of elongation | ELL | 80 kDa |
| | | EAP45 | 45 kDa |
| | | EAP30 | 30 kDa |
| | | EAP20 | 20 kDa |
| ELL2 | Increases rate of elongation | ELL2 | 84 kDa |
| CSB | Increases rate of elongation May link transcription and repair | CSB | ~165 kDa |
| Tat-SF1 | Increases rate of elongation | Tat-SF1 | 140 kDa |
| Elongator | Associated with elongating form of RNA polymerase II. Elp3 has histone acetyltransferase ability | Elp1 | 153 kDa |
| | | Elp2 | 90 kDa |
| | | Elp3 | 64 kDa |

[a]Human elongation factors are described here. The exception is Elongator, which has only been described in yeast thus far. Orthologues of many of these mammalian components exist in other organisms including yeast, *Drosophila*, *C. elegans*, and mouse.

that the action of TFIIH-associated helicases, and not the kinase, are essential for promoter escape (51, 140, 312, 393).

## Factors Influencing Elongation Processivity

TFIIS was originally identified by its ability to promote synthesis of long transcripts by purified RNA polymerase II. TFIIS enables RNA polymerase II to pass through various impediments to transcription, including intrinsic pause sites and nucleoprotein complexes (reviewed in 476). TFIIS interacts with arrested RNA polymerase II, activating an endoribonuclease activity. This nuclease cleaves the nascent mRNA, permitting repeated attempts at passing through the block to transcription (reviewed in 474).

Elongin (SIII) is a heterotrimer composed of the subunits elongin A, B, and C. Elongin A contains the stimulatory activity of the complex while the elongin BC complex interacts with numerous proteins, most notably the von Hippel-Lindau (VHL) tumor suppressor gene product (15, 136, 269, 277). Elongin was originally identified by its ability to stimulate the processivity of purified RNA polymerase II in vitro (52, 53).

The ELL gene product enhances the elongation rate of RNA polymerase II by its ability to suppress transient pausing (517). The human ELL gene undergoes frequent translocations with the MLL gene in acute myeloid leukemias. Full-length ELL (but not the ELL-MLL fusion) can also suppress RNA polymerase II, but this activity is blocked when ELL is present in a complex termed holo-ELL (514). Recently, the EAP30 subunit of holo-ELL has been cloned and shown to be similar to the *SNF8* gene in *S. cerevisiae* (500).

Cockayne Syndrome B (CSB) protein enhances elongation by RNA polymerase II in vitro (505). The yeast homologue of CSB, *RAD26*, suggests a link between transcription and transcription-coupled repair. CSB can be found in a complex with RNA polymerase II and may assist in both recruiting DNA repair proteins to the stalled elongation complex and allowing repair proteins access to the DNA (549, 551, 561, 577, 645).

Tat binds to the stem-loop structure located at the 5′ end of nascent HIV-1 transcripts and stimulates transcription by enhancing the processivity of RNA polymerase II. This enhancement is dependent on the CTD and sensitive to kinase inhibitors, strongly indicating that CTD kinases play a role in Tat-mediated stimulation. Tat associates with both P-TEFb (169, 370, 662, 664) and TFIIH (172, 437) to stimulate transcription of HIV. Affinity purification of Tat from HeLa extracts identified an RNA polymerase II holoenzyme that contained sufficient factors to enable Tat-mediated activation in vitro (125). In a related study, Tat function was examined in two systems, a reconstituted system and a nuclear extract, both of which were modified to be restrictive for Tat function (438). Under these conditions, Tat could only stimulate activation in the nuclear extract, implying that the nuclear extract contained factors lacking in the reconstituted system. Purification of this activity resulted in the identification of Tat-SF. Remarkably, Tat-SF includes RNA polymerase II and several other known Tat cofactors including

Tat-SF1 (663), hSpt5 (623), P-TEFb, and additional polypeptides. A Tat-SF-derived fraction lacking RNA polymerase II could impart Tat responsiveness to both RNA polymerase II and an SRB/Med-containing holoenzyme, neither of which is normally responsive to Tat.

Several factors have been identified that facilitate production of long transcripts from chromatin templates in vitro, indicating that elongation may be stimulated by remodeling of promoter-proximal histones. Two human factors, FACT and RSF, enable transcription by purified components on chromatin templates (334, 425). FACT interacts with histones (426), whereas RSF has ATP-dependent nucleosome remodeling and spacing activity (333, 334). The ARC/DRIP complex stimulates transcription specifically on chromatin templates, but the mechanism is unknown (401, 466).

### Additional Elongation Cofactors

Yeast RNA polymerase II molecules with highly phosphorylated CTDs can be isolated together with a complex called Elongator. Otero et al (430) separated chromatin-bound and soluble protein using high-speed centrifugation under high salt conditions. RNA polymerase II with phosphorylated CTD was found almost exclusively in the chromatin fraction, which also contained the Elongator complex. Elongator contains three subunits and requires hyperphosphorylated CTD for binding (430). Elongator has not been shown to directly stimulate the elongation rate, but genetic evidence supports a role for this factor in elongation. As previously noted, one of the elongator subunits contains histone acetyltransferase activity, suggesting one possible means of modifying the acetylation state across regions of actively transcribed DNA (612).

## RNA POLYMERASE II AND mRNA PROCESSING

Primary transcripts produced by RNA polymerase II are typically modified at both 5′ and 3′ ends, and are subjected to splicing. The 5′ ends of mRNAs are capped with a methylated guanosine triphosphate, and this modification is essential for further processing, localization, and translation (518, 579). The 3′ ends of mRNAs are cleaved and polyadenylated, and the 3′ polyA tract plays a role in transcript termination, transport, translation, and stability (387, 661). mRNAs can be edited after transcription by factors that modify, insert, and delete nucleotides (55). A system exists to proofread and degrade aberrant RNA molecules (126, 227, 229). The mature mRNA message must be conveyed to nuclear pores for export to the cytoplasm (404, 530, 535). Recent discoveries link several of these processing steps to the RNA polymerase II CTD and other components of the transcription apparatus.

### Links Between RNA Polymerase II and Capping

Nascent pre-mRNA is modified very early in its synthesis by addition of a 7-methylguanine triphosphate cap, in a 5′-5′ linkage. The phosphorylated form

of the RNA polymerase II CTD helps recruit the capping enzyme to the nascent transcript (98, 379). Transcripts made from RNA polymerase II molecules lacking CTDs are not capped, indicating that capping depends on an intact CTD (379). Mammalian and yeast guanylyltransferases bind phosphorylated RNA polymerase II and recombinant CTD, but not unphosphorylated CTD (98, 234, 379, 648). Binding to the phosphorylated CTD also affects the activity of components of the capping apparatus (97, 233).

### RNA Polymerase II and 3′ End Formation

Cleavage of the transcript at the polyadenylation site is dependent on the presence of an intact RNA polymerase II CTD (380). Both cleavage polyadenylation specificity factor (CPSF) and cleavage stimulation factor (CstF) can bind the CTD in crude extracts and copurify with RNA polymerase II complexes (380). The addition of recombinant CTD or intact RNA polymerase II can stimulate cleavage in vitro in the absence of transcription, suggesting that the CTD may stimulate the activity of cleavage factors or provide a scaffold for assembly of the complex (230).

The association of elements of the polyadenylation machinery with the RNA polymerase II CTD suggests a mechanism for efficient coordination of 3′ end formation and transcription termination. CPSF may accompany RNA polymerase during transcript elongation, allowing recognition of its cognate binding site on the transcript soon after its synthesis. A change in the association between CPSF and RNA polymerase II might cause the enzyme to switch to a less processive state, one that is primed for termination (reviewed in 37).

### RNA Polymerase II and Splicing

Efficient mRNA splicing may be physically and functionally linked to the RNA polymerase II CTD. Splicing is inhibited in vivo when genes are transcribed by RNA polymerase II molecules with truncated CTDs (380). Phosphorylated CTD can stimulate splicing in vitro (231). Interactions between activators, coactivators, and splicing factors can affect the use of splice sites downstream (123, 124, 178, 179, 324, 546). Immunoprecipitates of phosphorylated RNA polymerase II contain nuclear matrix components including members of the SR (Ser-Arg) and Sm (components of snRNP complexes that comprise the spliceosome) families of splicing factors (47, 278, 388, 394, 548, 590). Direct contacts have been documented between the CTD and other proteins with SR-rich domains including the SCAFs (SR-like CTD associated factor) (116, 446). Splicing is inhibited in vivo by overexpressing the CTD, consistent with the possibility that *trans*-acting splicing factors interact with this domain (135, 649).

## PERSPECTIVES

The past decade has seen considerable advances in our understanding of the chromatin template, activation and repression, the complexes that regulate chromatin structure, and RNA polymerase II and its initiation, elongation, and processing

cofactors. Several important insights have emerged. The chromatin template is modified by enzymes under the control of transcriptional activators and repressors to make it more or less available to the transcription apparatus. Transcriptional activators recruit a transcription initiation apparatus to promoters that is at least as large and complex as a ribosome, consisting of a mediator complex that provides an array of activator targets and the capacity to integrate multiple regulatory signals. The modular nature of the initiation apparatus provides an additional level of regulation, and can be modified in response to changes in a cell's environment. Transcription initiation, elongation, and mRNA processing are linked through the multiple functions of the RNA polymerase II CTD.

We can anticipate significant advances in transcription coming from several areas of investigation. It will be exciting to learn how cells coordinate the response to changing environments via multiple signal transduction pathways. It will be important to identify the requirements for components of the transcription apparatus at all genes in living cells. Detailed study of the regulation of individual genes will continue to improve our understanding of the mechanisms that have evolved to solve problems in combinatorial control, cell cycle timing, rapid response to environmental changes, and other specific regulatory challenges in cells. Additional valuable insights into the molecular mechanisms generally involved in gene regulation will come from structural studies.

It is now possible to envision the development of maps of transcriptional regulatory circuitry in cells. Genome-wide expression analysis with high-density DNA arrays has already been useful for identifying genes whose expression depends on individual components of the yeast transcription apparatus. The coupling of genome-wide expression analysis using high-density DNA arrays with mathematical and computational methods can produce models for transcriptional regulatory networks. Such models can be tested with a combination of genetic and genomic approaches, and the molecular mechanisms responsible for the regulation observed in networks can be identified with biochemical and molecular genetic approaches.



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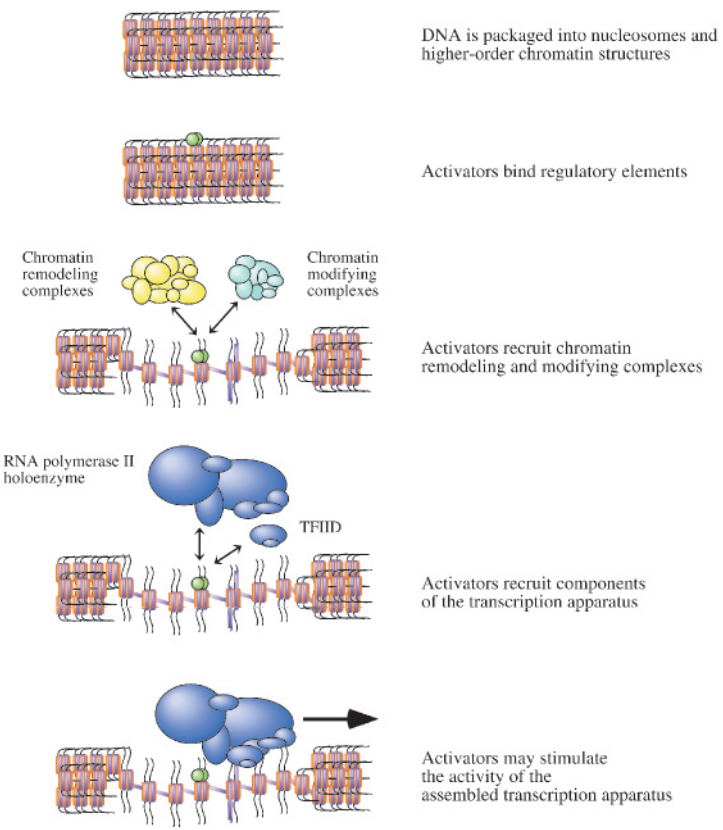


**Figure 1** A model for the role of activators in transcription initiation.
Several themes are featured. Transcriptional activators bind to specific DNA sequences, and the chromatin context of the DNA binding site can have a positive or negative influence on binding of the activator or the proteins it recruits to the promoter. Activators can bind and recruit chromatin remodeling and modifying complexes that influence local chromatin structure. One function of chromatin remodeling may be to increase the stability of the activator-DNA complex, and another to affect access of promoter sequences for binding of the transcription apparatus. Activators also bind and recruit the transcription initiation apparatus to promoters, probably through the concerted interactions of a few large complexes. Note that most of these processes are reversible, and are regulated by transcriptional repressors. Activators may also influence events subsequent to assembly of the initiation apparatus such as promoter clearance and RNA polymerase processivity. The diagram suggests an order to the process of gene activation, although it is not clear that this occurs at all promoters in vivo. The diagram shows a single activator bound at the promoter, but promoters typically contain multiple activator binding sites.

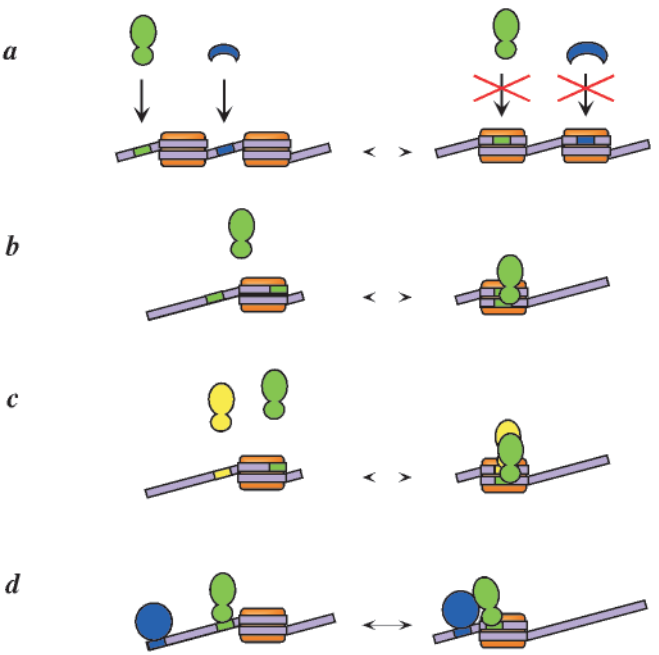


**Figure 2** Effects of nucleosomes on binding of activators and transcriptional machinery. (*a*) The packaging of DNA into nucleosomes is generally regarded as a block to transcription, presumably because the nucleosome interferes with binding of activators (*green*) or elements of the transcription machinery (*blue*). (*b*) Nucleosomes may serve a positive role in transcription by positioning two distinct DNA segments to create a complete binding site. (*c*) Nucleosomes may position independent activator binding sites to permit synergistic binding of activators. (*d*) Nucleosomes may alter the orientation or distance between factors, thereby stimulating interactions required for transcription.