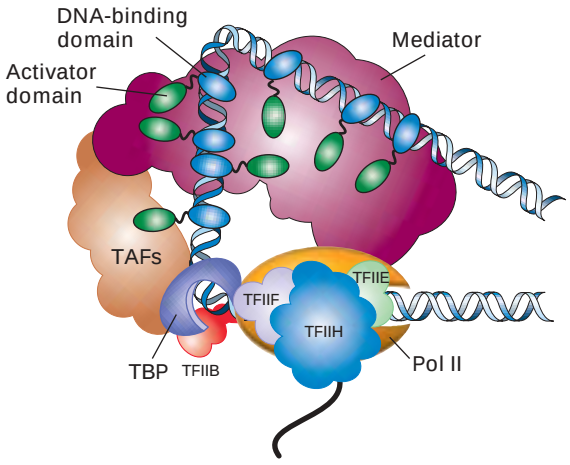
an activator bound to its cognate site in DNA and Pol II at a promoter. In addition, one of the mediator subunits has histone acetylase activity and may function to maintain a promoter region in a hyperacetylated state.

Experiments with temperature-sensitive yeast mutants indicate that some mediator subunits are required for transcription of virtually all yeast genes. These subunits most likely help maintain the overall structure of the mediator complex or bind to Pol II and therefore are required for activation by all activators. In contrast, other mediator subunits are required for activation of specific subsets of genes. DNA microarray analysis of gene expression in mutants with defects in these mediator subunits indicates that each such subunit influences transcription of ≈3–10 percent of all genes (see Figure 9-35 for DNA microarray technique).These mediator subunits are thought to interact with specific activation domains; thus when one subunit is defective, transcription of genes regulated by activators that bind to that subunit is severely depressed but transcription of other genes is unaffected. Consistent with this explanation are binding studies showing that some activation domains do indeed interact with specific mediator subunits.

Large mediator complexes, isolated from cultured mammalian cells, are required for mammalian activators to stimulate transcription by Pol II in vitro. Since genes encoding homologs of the mammalian mediator subunits have been identified in the genome sequences of *C. elegans* and *Drosophila*, it appears that most multicellular animals (metazoans) have homologous mediator complexes. About one third of the metazoan mediator subunits are clearly homologous to yeast mediator subunits (see Figure 11-35b). But the remaining subunits, which appear to be distinct from any yeast proteins, may interact with activation domains that are not found in yeast. As with yeast mediator, some of the mammalian mediator subunits have been shown to interact with specific activation domains. For example, the Sur2 subunit of mammalian mediator binds to the activation domain of a TCF transcription factor that controls expression of the *EGR-1* gene (see Figure 11-19). The function of this TCF activator in vivo normally is regulated in response to specific protein hormones present in serum. Mouse embryonic stem cells with a knockout of the *sur2* gene fail to induce expression of EGR-1 protein in response to serum, whereas multiple other activators function normally in the mutant cells. This finding implicates the mediator Sur2 subunit in the activating function of TCF.

The various experimental results indicating that individual mediator subunits bind to specific activation domains suggest that multiple activators influence transcription from a single promoter by interacting with a mediator complex simultaneously (Figure 11-36). Activators bound at enhancers or promoter-proximal elements can interact with mediator associated with a promoter because DNA is flexible and can form a loop bringing the regulatory regions and the promoter close together. Such loops have been observed in experiments with the *E. coli* NtrC activator and σ^{54}-RNA polymerase (see Figure 4-17). The multiprotein nucleoprotein complexes that form on eukaryotic promoters may comprise as many as 100 polypeptides with a total mass of ≈3 megadaltons (MDa), as large as a ribosome.



▲ FIGURE 11-36 **Model of several DNA-bound activators interacting with a single mediator complex.** The ability of different mediator subunits to interact with specific activation domains may contribute to the integration of signals from several activators at a single promoter. See the text for discussion.

Transcription of Many Genes Requires Ordered Binding of Activators and Action of Co-Activators

We can now extend the model of Pol II transcription initiation in Figure 11-27 to take into account the role of activators and co-activators. These accessory proteins function not only to make genes within nucleosomal DNA accessible to general transcription factors and Pol II but also directly recruit Pol II to promoter regions.

Recent studies have analyzed the order in which activators bind to a transcription-control region and interact with co-activators as a gene is induced. Such studies show that assembly of preinitiation complexes depends on multiple protein-DNA and protein-protein interactions, as illustrated in Figure 11-37 depicting activation of the yeast *HO* gene. This gene encodes a sequence-specific nuclease that initiates mating-type switching in haploid yeast cells (see Figure 11-28). Activation of the *HO* gene begins with binding of the SWI5 activator to an upstream enhancer. Bound SWI5 then interacts with the SWI/SNF chromatin-remodeling complex and GCN5-containing histone acetylase complex. Once the chromatin in the *HO* control region is decondensed and hyperacetylated, a second activator, SBF, can bind to several sites in the promoter-proximal region. Subsequent binding of the mediator complex by SBF then leads to assembly of the transcription preinitiation complex containing Pol II and the general transcription factors shown in Figure 11-36.