

of its C-terminal fragments, the resulting truncated proteins retained the ability to stimulate expression of a reporter gene in an *in vivo* assay like that depicted in Figure 11-16. Thus the internal portion of the protein is not required for functioning of GAL4 as a transcription factor. Similar experiments with another yeast transcription factor, GCN4, which regulates genes required for synthesis of many amino acids, indicated that it contains an ≈ 60 -aa DNA-binding domain at its C-terminus and an ≈ 20 -aa activation domain near the middle of its sequence.

Further evidence for the existence of distinct activation domains in GAL4 and GCN4 came from experiments in which their activation domains were fused to a DNA-binding domain from an entirely unrelated *E. coli* DNA-binding protein. When these fusion proteins were assayed *in vivo*, they activated transcription of a reporter gene containing the cognate site for the *E. coli* protein. Thus functional transcription factors can be constructed from entirely novel combinations of prokaryotic and eukaryotic elements.

Studies such as these have now been carried out with many eukaryotic activators. The structural model of eukaryotic activators that has emerged from these studies is a modular one in which one or more activation domains are connected to a sequence-specific DNA-binding domain through flexible protein domains (Figure 11-18). In some cases, amino acids included in the DNA-binding domain also contribute to transcriptional activation. As discussed in a later section, activation domains are thought to function by

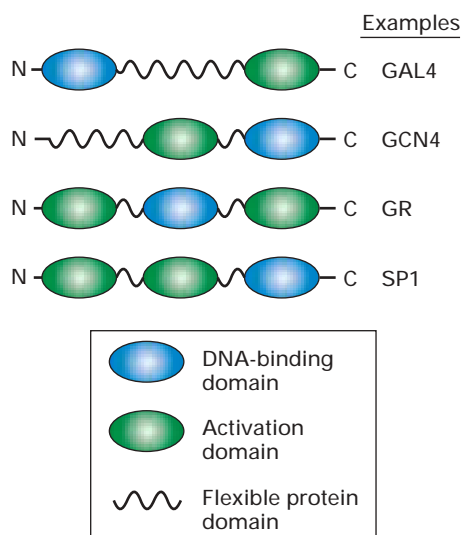
binding other proteins involved in transcription. The presence of flexible domains connecting the DNA-binding domains to activation domains may explain why alterations in the spacing between control elements are so well tolerated in eukaryotic control regions. Thus even when the positions of transcription factors bound to DNA are shifted relative to each other, their activation domains may still be able to interact because they are attached to their DNA-binding domains through flexible protein regions.

Repressors Are the Functional Converse of Activators

Eukaryotic transcription is regulated by repressors as well as activators. For example, geneticists have identified mutations in yeast that result in continuously high expression of certain genes. This type of unregulated, abnormally high expression is called **constitutive** expression and results from the inactivation of a repressor that normally inhibits the transcription of these genes. Similarly, mutants of *Drosophila* and *C. elegans* have been isolated that are defective in embryonic development because they express genes in embryonic cells where they are normally repressed. The mutations in these mutants inactivate repressors, leading to abnormal development.

Repressor-binding sites in DNA have been identified by systematic linker scanning mutation analysis similar to that depicted in Figure 11-10. In this type of analysis, mutation of an activator-binding site leads to decreased expression of the linked reporter gene, whereas mutation of a repressor-binding site leads to increased expression of a reporter gene. Repressor proteins that bind such sites can be purified and assayed using the same biochemical techniques described earlier for activator proteins.

Eukaryotic transcription repressors are the functional converse of activators. They can inhibit transcription from a gene they do not normally regulate when their cognate binding sites are placed within a few hundred base pairs of the gene's start site. Like activators, most eukaryotic repressors are modular proteins that have two functional domains: a DNA-binding domain and a **repression domain**. Similar to activation domains, repression domains continue to function when fused to another type of DNA-binding domain. If binding sites for this second DNA-binding domain are inserted within a few hundred base pairs of a promoter, expression of the fusion protein inhibits transcription from the promoter. Also like activation domains, repression domains function by interacting with other proteins as discussed later.



▲ FIGURE 11-18 Schematic diagrams illustrating the modular structure of eukaryotic transcription activators.

These transcription factors may contain more than one activation domain but rarely contain more than one DNA-binding domain. GAL4 and GCN4 are yeast transcription activators. The glucocorticoid receptor (GR) promotes transcription of target genes when certain hormones are bound to the C-terminal activation domain. SP1 binds to GC-rich promoter elements in a large number of mammalian genes.



The absence of appropriate repressor activity can have devastating consequences. For instance, the protein encoded by the *Wilms' tumor (WT1)* gene is a repressor that is expressed preferentially in the developing kidney. Children who inherit mutations in both the maternal and paternal *WT1* genes, so that they produce no functional WT1 protein, invariably develop kidney tumors