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Transcriptional Regulation in Mammalian Cells by Sequence-Specific DNA Binding Proteins

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The cloning of genes encoding mammalian DNA binding transcription factors for RNA polymerase II has provided the opportunity to analyze the structure and function of these proteins. This review summarizes recent studies that define structural domains for DNA binding and transcriptional activation functions in sequence-specific transcription factors. The mechanisms by which these factors may activate transcriptional initiation and by which they may be regulated to achieve differential gene expression are also discussed.

INITIATION OF MESSENGER RNA (MRNA) SYNTHESIS IS A primary control point in the regulation of differential gene expression. Cells respond to intra- and extracellular cues by turning certain genes on or off and by modulating the extent of transcription of active genes. In higher eukaryotes, transcriptional changes in the context of a developmental program can have profound long-term consequences. Although the mechanisms and biochemical pathways by which cells integrate physiological cues to bring about appropriate transcriptional changes are still largely unknown, it is clear that the frequency of initiation of mRNA synthesis depends ultimately on factors that interact with specific

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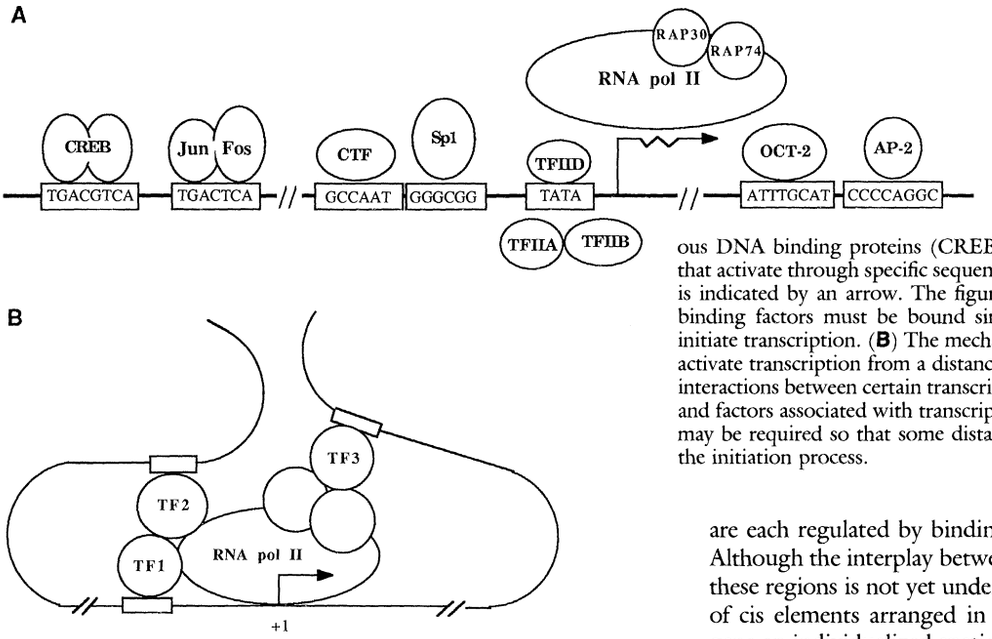

Fig. 1. Features of the transcriptional control region for a mammalian protein-coding gene. (**A**) A hypothetical array of cis elements that constitute the promoter and enhancer regions of a gene transcribed by pol II. Proteins that associate at these control regions are symbolically represented and include pol II, TFIIA, TFIIB, TFIID, RAP30, and RAP74 of the general transcriptional machinery (*2*), and various DNA binding proteins (CREB, Jun, Fos, CTF, Sp1, OCT-2, and AP-2) that activate through specific sequence elements. The transcription initiation site is indicated by an arrow. The figure is not meant to imply that all the DNA binding factors must be bound simultaneously as depicted here in order to initiate transcription. (**B**) The mechanism or mechanisms by which cis elements activate transcription from a distance are unknown, but specific protein-protein interactions between certain transcription factors (TFs) bound to distal elements and factors associated with transcription initiation complexes at RNA start sites may be required so that some distally bound factors can participate directly in the initiation process.

elements in gene promoters. The purpose of this review is to survey recent progress toward understanding the structure, function, and regulation of nuclear proteins that regulate RNA polymerase II (pol II) transcription initiation by binding to cis elements in mammalian genes.

cis Elements That Bind Transcription Factors

In contrast to the paradigm of prokaryotic cis elements at −10 and −35 nucleotides relative to the transcription initiation site, an assortment of regulatory elements for pol II transcription can be scattered both upstream and downstream of the RNA start site for a gene (*1, 2*). Systematic mutational analyses reveal that each gene in an animal cell has a particular combination of positive and negative regulatory cis elements that are uniquely arranged as to number, type, and spatial array (Fig. 1). These elements are binding sites for sequence-specific transcription factors that activate or repress transcription from the gene. Usually cis elements are arrayed within several hundred base pairs from the initiation site, but some elements can exert control over much greater distances (1 to 30 kb). The control region in the immediate vicinity of a transcription start site is called the promoter; regions that regulate a promoter from a distance and in an orientation-independent fashion are called enhancers (*3*). Some promoter elements, such as TATA, GC, and CCAAT boxes, are common to many genes transcribed by pol II. A large number of less common elements have been implicated in specialized types of signal-dependent transcriptional regulation, such as in response to heat shock, hormones, and growth factors. The importance of a particular cis element can vary greatly in different cell types and in response to physiological signals, presumably because the DNA binding factor or factors that recognize the element vary in abundance or in ability to function in different tissues and under different circumstances (*4*). Overlapping or superimposed binding sites for multiple factors can result in different positive and negative factors competing for sites; in some cases, synergistic effects that are dependent on strict spacing between adjacent cis elements have been observed (*5*). Various types of silencer elements can block activity of cis-linked enhancers (*6*). In addition, some genes have multiple transcription initiation sites that are each regulated by binding sites for different sets of factors (*7*). Although the interplay between the various factors that may bind to these regions is not yet understood, it is believed that combinations of cis elements arranged in unique configurations confer on each gene an individualized spatial and temporal transcription program.

Sequence-Specific DNA Binding Proteins

Factors that bind to cis-element DNA sequences can be detected in cell extracts and identified according to their sequence specificities in various in vitro DNA binding assays (*8*). These low-abundance proteins can be purified from nuclear extracts by sequence-specific DNA–affinity chromatography in order to obtain material for biochemical experiments and for protein sequence analysis (*9*); their binding properties have also been used as a means to screen expression libraries for complementary DNA (cDNA) clones encoding them (*10*). Structure-function studies of cloned mammalian DNA binding transcription factors have included the general strategy of dissecting protein structure by deletion analysis, in order to compare the biochemical activities of mutant versions of the factor. Most commonly, the DNA binding properties of wild-type and mutant factors are assayed in vitro, and the transcriptional activities are assayed by expressing the factor transiently in tissue culture cells and measuring the transcription from cotransfected reporter gene promoters containing binding sites for the factor. Such approaches have shown that different DNA binding transcription factors are composed of a surprising variety of usually separable DNA binding and transcriptional activation domains (also see Table 1).

DNA Binding Domains of Transcription Factors

The specific DNA binding activities of several mammalian factors have been localized to relatively small subregions consisting of 60 to 100 amino acids. These studies have demonstrated that a DNA binding domain is necessary but not sufficient for transcriptional activation. The amino acid sequences of four different types of DNA binding domains of mammalian transcription factors are shown in Fig. 2A.

Zinc finger motifs were originally identified as DNA binding structures in the RNA polymerase III transcription factor TFIIIA, which binds to the internal control region of the 5*S* RNA gene (*11*). Since then, at least two types of zinc fingers have also been found in DNA binding factors that participate in transcription mediated by

pol II (*12*). TFIIIA-like zinc fingers are present in the mammalian transcription factor Sp1 (*13, 14*) and in a variety of other regulatory proteins found in higher and lower eukaryotes. This type of zinc finger motif consists of ~30 amino acids with two cysteine and two histidine residues that stabilize the domain by tetrahedrally coordinating a Zn^{2+} ion. A region of ~12 amino acids between the invariant cysteine-histidine pairs is characterized by scattered basic residues and several conserved hydrophobic residues. Mammalian Sp1, which activates transcription by binding selectively to GC box cis elements, contains three tandem zinc fingers at its COOH-terminus; these are necessary and sufficient for binding and require zinc to do so. A second class of zinc finger motifs is exemplified by the DNA binding domains of steroid hormone receptors. This motif uses two pairs of cysteines rather than the cysteine-histidine arrangement typical of Sp1. The rat glucocorticoid receptor requires zinc for DNA binding activity, and substitution mutations that alter key cysteine residues in the receptor result in loss of function (*15*). Cysteine-containing motifs with varying degrees of similarity to zinc fingers of Sp1 and steroid hormone receptors have also been noted in other prokaryotic and eukaryotic proteins, not all of which are thought to be DNA binding proteins (*16*). These residues are widely used to coordinate metal ions and to form disulfide bridges in other types of protein structures, both within and between proteins.

The zinc finger DNA binding proteins characterized so far exhibit a variety of DNA sequence specificities. So, although the highly conserved amino acids in the finger motif may confer a structural framework for the binding domain, the determinants of binding specificity must lie elsewhere. For example, the slightly different DNA binding specificities of the progesterone and estrogen receptors have been found to be dependent on differences in several nonconserved amino acids at the base of the finger region (*17*).

A second type of DNA binding domain is the homeodomain (HD). This domain, which encompasses ~60 amino acids, was first identified as a conserved protein segment in several regulators of *Drosophila* embryogenesis and was soon found in genes of vertebrate organisms as well (*18*). The HD primary sequence is distantly related to the helix-turn-helix DNA binding structures of prokaryotic repressors, and the positions that are most conserved between HDs of different proteins are basic and hydrophobic residues. Because of the genetically identified regulatory relationships that exist between many of the *Drosophila* HD-containing proteins that regulate embryogenesis, it was anticipated that HD-containing proteins might be DNA binding factors that regulate transcription of genes (*18*). The finding that some HD-containing proteins bind to certain AT-rich elements in control regions of their own genes or those of other HD-containing factors, or both, supported this idea (*19*). In addition, in vitro transcription experiments, and transient cotransfection of reporter genes and genes encoding various HD-containing developmental regulatory proteins into *Drosophila* tissue culture cells suggested that some of the genetically defined effects that these proteins have on gene expression are at the level of transcription (*20*).

Conclusive evidence that HD-containing proteins can bind and directly activate transcription of target genes came from biochemical and in vivo studies of three recently cloned mammalian transcription factors. HD motifs are found in the octamer binding factors OCT-1 (*21*) (also known as OTF-1, NF-A1, and NFIII), OCT-2 (*22*) (also known as OTF-2 and NF-A2), and the pituitary-specific factor Pit-1 (GHF-1) (*23*). The presence of an HD in OCT-1 shows that this structure is not restricted to developmental regulators, as OCT-1 appears to be expressed in all mammalian cells and activates transcription of target genes that include histone H2B. The DNA binding domains of Pit-1, OCT-1, the lymphoid cell–specific OCT-2, and Unc-86, a developmental regulatory protein of the nematode *Caenorhabditis elegans* (*24*), constitute a novel subclass within the HD-containing protein family; these factors each contain a conserved bipartite domain of ~160 amino acids, termed the POU domain (*21*), which consists of an HD and a second region, the POU box.

A third type of DNA binding domain was first described for the mammalian enhancer binding protein C/EBP (*25*). A search for proteins with similarities to C/EBP identified several transcription factors that share a bipartite region of primary sequence similarity consisting of a highly conserved stretch of ~30 amino acids with a substantial net basic charge immediately followed by a region containing four leucine residues positioned at intervals of seven amino acids. The latter segment, named the "leucine zipper" by McKnight and colleagues, is required for dimerization and for DNA binding (*25*). It is believed that dimerization of proteins in this group, which includes mammalian C/EBP, Jun, Fos, and CREB, is stabilized by hydrophobic interactions between closely apposed α-helical leucine repeat regions of the two subunits (*25–27*). The basic region adjacent to the leucine motifs seems to be necessary for DNA binding but not for dimerization, and both subunits appear to contribute their basic regions to form the DNA binding domain (*26*). Other proteins, including Myc and OCT-2, contain leucine repeat motifs without the conserved basic region found in the C/EBP-like factors. The leucine region in Myc is required for tetramerization and for transformation of primary cells (*28*). As mentioned above, OCT-2 has a homeodomain DNA binding structure; the function of the leucine repeat region is not known (*22*).

The DNA binding domain of the transcription-replication factor CTF/NF-I has been localized to the NH_2-terminal third of the protein by deletion analysis (*29, 30*). This region could form an α-helical structure and has a high density of basic amino acids consistent with a DNA binding structure, but has no features characteristic of binding domains associated with zinc fingers, HDs, or leucine zippers. The primary sequences of several other recently cloned mammalian transcription factors indicate that the number of DNA binding domain types is not limited to the four just described. For example, factors AP-2 (*31*) and SRF (the serum-response factor) (*32*) contain no obvious similarities to the binding domains described above, nor are they similar to each other.

Transcriptional Activation Domains

Transcriptional activation functions of DNA binding factors depend on regions of as few as 30 to 100 amino acids that are separate from the DNA binding domain. Factors often have more than one activation domain, and several apparently unrelated structural motifs have been identified that confer these functions (Fig. 2B). Different types of activation domains have been exchanged and paired with different DNA binding domains to produce chimeric transcription factors. The first defined activation regions in eukaryotic transcription factors were identified by studies of the yeast factors, GAL4 and GCN4 (*33, 34*). The activation domains of these factors consist of relatively short stretches of amino acids with apparently only two features in common: they are regions with significant negative charge and can form amphipathic α-helical structures. GAL4 has two separate acidic domains, which, despite their lack of obvious sequence homology, seem to be functionally redundant; each retains significant activity when placed at various positions relative to the DNA binding domain. When linked to a heterologous DNA binding domain, they can activate transcription of reporter genes with a binding site for the heterologous factor in yeast and in cells of a variety of higher organisms (*33*). One of two transcriptional activation domains identified in the glucocorticoid

hormone receptor fits the description of acidic α-helix (*35*). Similarly, there appears to be some correlation between activation and negatively charged α-helical regions in the AP-1/Jun transcription factors (*36, 37*). It has been proposed that acidic activation domains may facilitate transcription initiation by interacting in a relatively nonspecific manner with a general component of the initiation complex, such as the TATA-binding TFIID or possibly pol II itself (*33, 38*). Consistent with this idea, some acidic domains have been shown to stabilize a complex with TATA-binding factor on promoters in yeast and mammalian cells (*39*).

Deletion analysis of the transcription factor Sp1 has revealed four separate regions that contribute to transcriptional activation (*14*); all lie outside the zinc finger DNA binding domain. The two most potent activation domains contain ~25% glutamine and very few charged amino acid residues. A third activation domain, adjacent to the zinc fingers, is ~30 amino acids long and has a net positive charge. The COOH-terminal 30 amino acids of the protein constitute a fourth domain. The latter two regions display no obvious similarity to each other or to the glutamine-rich activation domains in Sp1. Inspection of other established or suspected transcription factors reveals glutamine-rich regions in *Drosophila* Antennapedia, Ultrabithorax, and Zeste proteins (*14*); in yeast HAP1, HAP2, and GAL11 (*40*); and in mammalian OCT-1 (*21*) and OCT-2 (*22*), Jun (*36*), AP-2 (*31*), and SRF (*32*), although the activation functions in these proteins have not yet been systematically localized. A glutamine-rich stretch of 145 amino acids from Antennapedia can partially substitute for activation domains of Sp1 when linked to the Sp1 zinc fingers (*14*). Thus, Antennapedia may mediate transcriptional activation through this glutamine domain in its natural context, and like acidic domains, glutamine domains may be interchangeable. Except for glutamine content, there are no obvious sequence homologies between the Antennapedia segment and the activation domains found in Sp1. This is reminiscent of findings on acidic activation domains, which also exhibit a lack of primary sequence similarities. The amino acid sequences of the activation domains as well as the zinc fingers of Sp1 are highly conserved between human and frog relative to other portions of the protein, implying that there has been selective pressure during evolution to maintain their specific structures (*41*).

A third type of activation domain has been identified in CTF/NF-I (*30*). A proline-rich (20 to 30%) domain in the COOH-terminus of CTF activates transcription when linked to various DNA binding domains including the zinc fingers of Sp1. Regions rich in proline, an α-helix breaker, have also been noted in many other mammalian transcription factors, including AP-2 (*31*), Jun (*37*), OCT-2 (*22*), and SRF (*32*).

The three different primary sequence motifs that characterize the activation domains identified thus far (that is, acidic, glutamine-rich, and proline-rich) (Fig. 2B) are likely to represent regions that function by contacting other proteins. The idea that all activation domains directly contact and stabilize the binding of the same general transcription factor, such as TATA-binding TFIID, seems

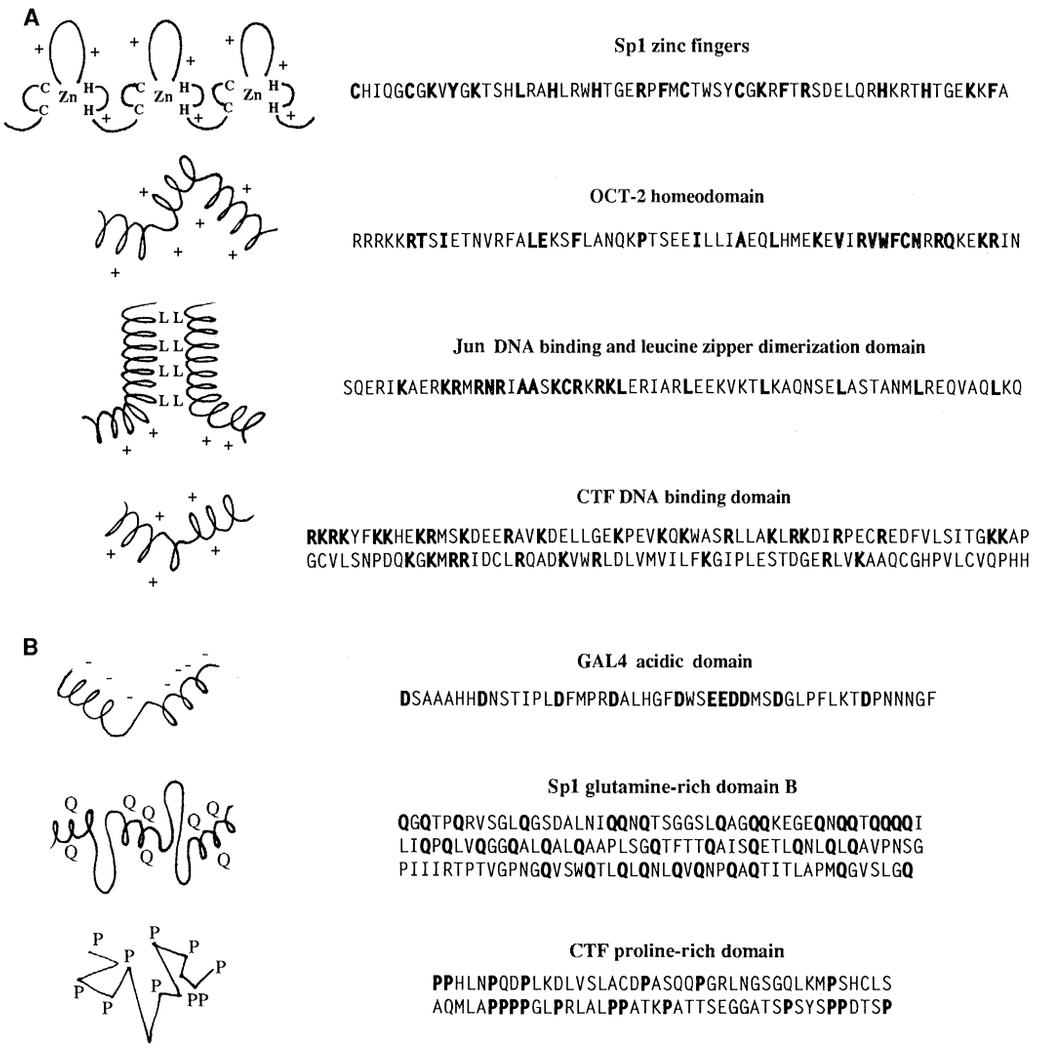

Fig. 2. DNA binding and transcriptional activation domains in transcription factors for pol II. (**A**) Four structural domains used for sequence-specific DNA binding by mammalian transcription factors are represented on the left and accompanied on the right by the amino acid sequence [in single-letter code (*71*)] of the delimited domain. Amino acid residues that are highly conserved between different zinc finger, HD, or leucine repeat–associated DNA binding domains are designated by boldface type. Positively charged amino acids within the DNA binding domain of transcription factor CTF/NF-I are also in boldface type. The figures on the left are symbolical representations and are not based on structural studies. (**B**) Three types of protein domains responsible for transcriptional activation by DNA binding factors. Amino acid residues that characterize the three domain types: acidic (D, E), glutamine-rich (Q), and proline-rich (P) are highlighted by boldface type.

unlikely, unless TFIID can accommodate multiple interaction surfaces. At least five general transcription factors are required for initiation by pol II (*2*), and it is not difficult to envision that sequence-specific transcription factors have different types of activation domains to contact different general factors or different subunits of pol II. It has been proposed that some eukaryotic transcription activators could function by interacting with the threonine- and serine-rich COOH-terminal portion of the largest subunit of pol II (*42*). This region may constitute a relatively nonspecific interactive surface, rich in hydroxyl groups, through which activation domains of one or more transcription factors bound to the DNA template could engage the polymerase molecule by hydrogen bonding. Phosphorylation of these hydroxyl groups may be a possible mechanism for disengaging polymerase from the factors to allow initiation of transcription and elongation.

The 5′ flanking regions of transcriptionally active genes can often be structurally distinguished as nucleosome-free deoxyribonuclease-hypersensitive zones occupied by nonhistone, DNA binding proteins (*43*). Experiments indicate that formation and stabilization of transcription initiation complexes with sequence-specific transcription factors can competitively exclude histones and prevent the inhibitory effects of nucleosome assembly on transcription in vitro (*44*). It is likely that there are multiple mechanims by which initiation complexes are stabilized in vivo, and that different activation domains of DNA binding transcription factors underly some of these mechanisms (Fig. 3). Specific associations between regulatory regions of active genes and nuclear scaffold proteins have also been demonstrated (*45*); perhaps some activation domains contact proteins of the nuclear matrix. These interactions could facilitate transcription if, for example, they serve to tether genes to nuclear regions with locally high concentrations of other essential transcription and processing factors.

Families of Transcription Factors with Related DNA Binding Specificities

Like many of the genes they control, DNA binding transcription factors have increased in number and diversity during evolution by processes such as gene duplication, divergence, and exon shuffling. The related DNA sequence specificities of several groups of mammalian transcription factors suggest that expression, regulated post-translational modifications, binding affinity, binding site context, and interactions with other regulatory proteins must play an important role in focusing the action of these factors to their respective target gene promoters.

Proteins of the AP-1 family can be purified from nuclear extracts by DNA affinity chromatography with oligonucleotides bearing the sequence TGACTCA (*36, 46*). In mammals, these proteins are encoded by multiple genes including c-*jun*, *jun*B, *jun*D, *fos*, and *fra*-1 (*47*). Factors that bind to AP-1 sites are also found in *Drosophila* and yeast (*48*). The shared DNA binding specificity of AP-1 family members is reflected in the conservation of their leucine zipper–associated DNA binding domains; regions outside the DNA binding domain may be unrelated or have diverged substantially in different family members. Recent studies on Fos and Jun proteins indicate that homo- and heterodimerization of AP-1 proteins by means of their leucine-zipper regions may play an important role in transcriptional regulation by creating a variety of transcription factors with different functional properties. The AP-1 protein c-Jun is now recognized to be identical to p39, a protein first identified by its stable association with Fos in cell extracts (*47*). It has been shown that c-Jun and Fos, when synthesized together in in vitro translation systems, form heterodimers that can bind to AP-1 sites; and c-Jun, but not Fos, when synthesized alone, forms homodimers that bind AP-1 sites (*26*). Analyses of point mutations in the leucine repeats of Fos indicate that this region is required for heterodimerization between Fos and c-Jun and for the oncogenic transformation function of Fos (*26, 49*). The in vitro synthesis of c-Jun–Fos and c-Jun–FRA-1 heterodimers, as well as hetero- and homodimers between the various Jun proteins, suggests that transcriptional regulation of genes with AP-1 sites may be complex (*47, 50*). The promoter of the *fos* gene itself provides an example of this potential complexity. Most of the AP-1 proteins can be induced by serum [and also by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)] in different cell types; but the kinetics and cell type specificity of mRNA accumulation and protein turnover differ among family members (*47, 51*). Induction of Fos mRNA by serum stimulation appears to be autoregulated in mammalian cells by negative feedback control, and the positive and negative phases of induction are both controlled through a region of the *fos* promoter that includes an AP-1 site (*52*).

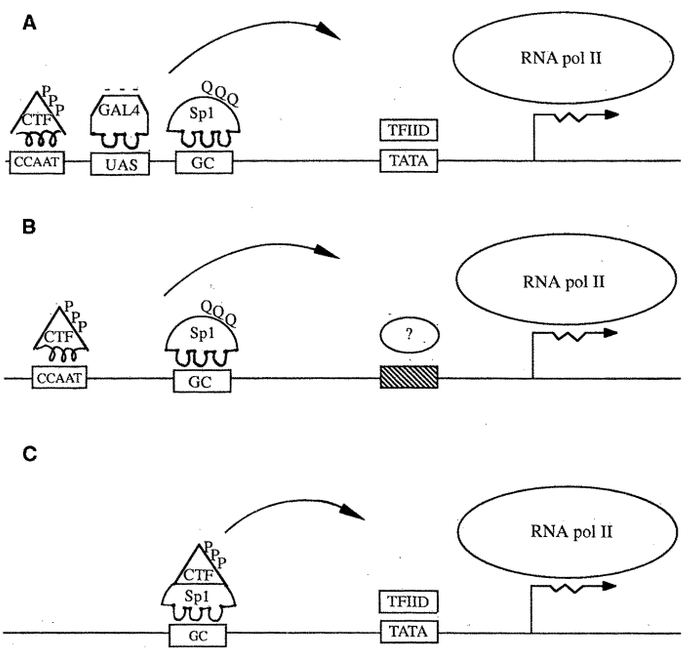

Fig. 3. Potential mechanisms of transcriptional activation. (**A**) At least three distinct types of activation domains: acidic, proline-rich, and glutamine-rich have already been identified. These different domains seem to be comparably efficient in activating various types of promoters; however, their mechanisms of action, whether similar or distinct, are unknown. It has been proposed that DNA binding factors may use their activation domains to contact components of the general transcriptional apparatus. TFIID, the TATA-binding factor, is one of the target proteins proposed for acidic activation domains (*38, 39*). Transcription factors that activate via glutamine-rich or proline-rich domains can also function in the context of TATA box–containing promoters, suggesting either that TFIID can accommodate different types of activation domains, or that some activation domains function without directly interacting with TFIID. (**B**) Some genes do not appear to have TATA boxes and may rely on alternative mechanisms of transcriptional activation. Different activation domains could conceivably target initiation complex components other than TFIID, such as subunits of pol II or general factors such as TFIIA, TFIIB, RAP30, or RAP74. Some DNA binding proteins may stimulate transcription by less direct mechanisms related to nucleosome phasing or nuclear localization. (**C**) A promoter that depends on a specific upstream cis element such as the GC box, normally recognized by the transcription factor Sp1 containing a glutamine-activation domain, can also be activated by a chimeric protein consisting of the Sp1 DNA binding domain linked to a heterologous CTF proline-rich activation domain. This and other experiments with chimeric transcription factors underscore the relative autonomy of DNA binding and activation domains in transcription factors.

A DNA element that confers induction by adenosine 3′,5′-monophosphate (cAMP) to some mammalian gene promoters (CRE, or cAMP-responsive element) differs from the AP-1 binding site by insertion of a single nucleotide (*53*). A CRE-binding protein (CREB) encoded by cDNA clones isolated from human JEG-3 and rat PC12 cells contains a region that is 61% identical to the positively charged DNA binding domain of c-Jun (*54*) and is associated with a leucine-zipper motif. Other regions of CREB are apparently unrelated to known AP-1 proteins. Both AP-1 and CREB proteins can each bind weakly to the other's defined recognition sequence; however, it is not yet known whether the two types of proteins can form heterodimers that combine their respective functional properties.

Several studies comparing factors with very similar DNA binding specificities demonstrate that sequence specificity is certainly not the only consideration that determines the promoter selectivity of a transcription factor. For example, the mammalian factors OCT-1 and OCT-2 have closely related HD-containing DNA binding domains and bind with apparently identical specificities to octamer sites (ATTTGCAT) in vitro. The OCT-1 factor, which is expressed in all cell types, is normally not able to activate transcription of lymphoid cell–specific immunoglobulin (Ig) genes with octamer sites (*55*). Selective activation of these genes by OCT-2 could be mediated by functional domains unique to OCT-2 that allow it to interact productively with other proteins associated with Ig promoter initiation complexes. The OCT-2 factor contains a leucine-repeat motif not found in OCT-1, but it is not known whether dimerization distinguishes the two factors or is otherwise involved in their regulation. Like the OCT proteins, HD-containing proteins from many different sources seem to have similar DNA binding site specificities; thus, studies on OCT proteins may suggest mechanisms by which the promoter selectivity of HD-proteins is regulated.

Another family of mammalian transcription factors recognizes CCAAT elements found in many gene promoters and enhancers. Differential splicing of mRNA from the CTF/NF-I gene in HeLa cells produces several CCAAT-binding proteins that each have different versions of a COOH-terminal, proline-rich transcriptional activation domain; these could conceivably be regulated to activate transcription in different contexts (*29, 30*). The CCAAT-binding protein CP1 consists of two components, A and B, which can be functionally complemented by yeast transcription factors HAP2 and HAP3 (*56*). CP1 and CTF can be distinguished on the basis of binding affinities for different permutations of the CCAAT element; the two factors are antigenically unrelated, and there are no amino acid sequence similarities between CTF and HAP2 or HAP3 proteins. Thus, CTF and CP1 are probably not encoded by closely related genes.

Regulation of Transcription Factor Activities by Posttranslational Modification

When signal-dependent transcriptional induction is unaffected by treatment of cells with protein synthesis inhibitors, it is assumed that all the protein components that transduce the stimuli and ultimately

Table 1. Features of selected DNA binding transcription factors.

Factor	Binding site (5′–3′) and size*(kD)	Features	References
GR†	GGTACAN$_3$TGTTCT D = 87.5 A = 94 (rat liver)	Glucocorticoid receptor or glucocorticoid-dependent transcription factor. Superfamily includes steroid and thyroid hormone receptors and retinoic acid receptors. Hormone-free receptor bound to hsp90 in cytoplasm. DNA binding domain with zinc finger of C_4 type.	(*15, 35, 61, 63*)
Sp1	GGGCGG D = 80 A = 95–105 (HeLa)	D = 80 kD, using tentatively assigned translation start. DNA binding domain with three zinc fingers of C_2H_2 type. Multiple activation domains; strongest have high percentage of glutamine. No known Sp1 homolog below vertebrates. O-Glycosylated.	(*13, 14, 41, 60*)
CTF/NF-I	GCCAAT D ≤ 55 A = 52 (HeLa)	Differential splicing results in CTFs with alternative proline-rich transcriptional activation domains. Antigenically unrelated to multisubunit CCAAT-binding CP1. DNA binding domain sufficient for replication function.	(*29, 30, 56*)
c-Jun	TGAC/GTCA D = 36 A = 39 (HeLa)	Member of family of proteins that bind AP-1 sites, the latter confer TPA inducibility. c-Jun can form heterodimers with Fos and other AP-1 family members through leucine zipper motifs. Jun and Fos homologs in yeast and *Drosophila*.	(*26, 36, 37, 46–52*)
C/EBP	TGTGGAAAG D = 42; A = 42 (rat liver)	First described leucine zipper–associated DNA binding domain; leucine zipper is required for dimerization.	(*25*)
AP-2	CCCCAGGC D = 48; A = 50–52 (HeLa)	Binding and transcriptional activities inhibited by SV40 T antigen in vitro. AP-2 site confers TPA and cAMP inducibility. Retinoic acid induces AP-2 mRNA in NT2 teratocarcinoma cells.	(*31, 70*)
CREB	TGACGTCA A = 38; D = 35 (JEG-3) A and D = 43 (PC12)	Binding site confers cAMP inducibility and E1A transactivation. Phosphorylation promotes dimerization, DNA binding, and transcriptional activity in vitro. Leucine zipper–associated basic domain is 61% identical to c-Jun DNA binding domain.	(*53, 54*)
OCT-1	ATTTGCAT D = 76 A = 90–100 (HeLa)	Apparently ubiquitous in mammalian cells. Same binding specificity in vitro as OCT-2 on octamer sequences of histone H2B and Ig κ gene-promoters. POU domain homology shared with OCT-2, Pit-1, and Unc-86; bipartite POU domain includes POU-box and HD.	(*21, 23, 24, 55*)
OCT-2	ATTTGCAT D = 51 A = 59–62 (BJAB)	Expressed mainly in B and T lymphocytes; activates Ig genes with octamer binding sites. Has leucine zipper. HD-POU homology shared with OCT-1 and others.	(*22, 55*)
SRF	GATGTCCATA-TTAGGACATC D = 51.6; A = 67 (HeLa)	Binds to serum response element (SRE). SRE mediates responsiveness to serum, epidermal growth factor, TPA, and insulin. Dimerization and DNA binding domain has no leucine zipper or HD.	(*32*)

*Size is approximate molecular mass: deduced from DNA sequence (D) or apparent from SDS-polyacrylamide gels (A). †Member of steroid receptor family.

mediate the response through the target cis element or elements in the responding gene already exist in the cell. The cell surface receptor–mediated generation of second messenger molecules, such as cAMP and Ca^{2+}, and the subsequent activation of protein kinases and phosphatases (*57*) is a widespread mechanism of signal transduction in mammalian cells. Many transcription factors are phosphoproteins and their functions could therefore conceivably be regulated by phosphorylation-dephosphorylation events.

The genes encoding heat shock proteins and the mechanism of their transcriptional induction by hyperthermia and other types of stress are highly conserved in eukaryotes. The heat shock element (HSE) in promoters of these genes is recognized by a DNA binding transcription factor, HSF or HSTF (*58, 59*). Increased transcription of yeast heat shock genes correlates with increased phosphorylation of HSF that is already bound to heat shock gene promoters. It has been proposed that the negatively charged phosphate groups may activate transcription by a mechanism related to that used by the acidic domains of transcription factors such as yeast GAL4 and GCN4 (*59*). Heat induction of human and *Drosophila* heat shock genes, in contrast to yeast, involves an apparent increase in the binding of their respective HSFs to DNA (*58*); whether phosphorylation is involved in heat shock induction by these factors is not known. Evidence also supports a role of phosphorylation in regulating the activity of mammalian CREB factor (*53, 54*). CREB, which has a leucine zipper–associated DNA binding domain, exists as a mixture of transcriptionally active dimers and relatively inactive monomeric forms in equilibrium; phosphorylation shifts this equilibrium in favor of dimers.

Glycosylation has also been identified as a feature common to many transcription factors for pol II (*60*). Purified Sp1 from HeLa cells is highly glycosylated, containing, on average, ten O-linked *N*-acetylglucosamine sugars per molecule. Treatment with wheat germ agglutinin specifically inhibits the transcriptional activity of Sp1 in vitro, without altering its ability to bind DNA; however, the relevance of this modification for regulation in vivo is unknown. Glycosylation occurs on serine and threonine residues, and as these residues can also be sites for phosphorylation by protein kinases, the two types of modifications could be mutually exclusive.

Regulation by Protein-Protein Interactions

The formation and dissociation of protein-protein complexes is an integral part of the regulation of many cellular processes. Two examples of signal-dependent dissociation of protein complexes involved in regulating the activities of DNA binding transcription factors are found in the transformation of steroid receptors by hormone binding (*61*) and the activation of the Ig κ light chain enhancer-binding protein NF-κB by phorbol ester (for example, TPA) treatment (*62*). In both cases, in the absence of inductive signals, the inactive transcription factor is found in the cytoplasm in association with another protein. Neither case of activation requires new protein synthesis. Inactive steroid receptors associate with the heat shock protein, hsp90, which is abundant in the cytoplasm. As a result of hormone binding to the receptor protein, the complex of the receptor and hsp90 dissociates and nuclear localization signals within the receptor direct it into the nucleus, where it binds to and activates (or represses) transcription of genes with the appropriate response elements. The inhibitory effect of hsp90 on the unliganded receptor is mediated through the steroid binding domain of the receptor; the latter is sufficient to confer glucocorticoid-dependent regulation on other transcription factors (*63*). The transcription factor NF-κB is regulated by association with a cytoplasmically located inhibitor, IκB (*62*). In contrast to the heat-labile steroid receptor–hsp90 interaction, the association between NF-κB and IκB appears highly specific, and free IκB is not found in significant amounts in the cytoplasm. It is postulated that TPA induction leads to changes in IκB, rather than in NF-κB. Although initially believed to be expressed exclusively as a lymphoid-specific transcription factor, NF-κB is also found in the cytoplasms of nonlymphoid cell types, such as HeLa cells; NF-κB can be apparently activated by TPA-induction in these cells without concomitant transcriptional activation of lymphoid genes that contain the NF-κB binding site. Thus, additional components must play a part in forming active transcriptional initiation complexes at these genes.

Many viral early gene products are specialized transcription regulators that activate or repress transcription of specific viral and cellular genes without directly binding to target promoters (*64*). Evidence supports the idea that the effects of viral trans-activators are mediated through protein-protein interactions with DNA binding transcription factors. The E1A protein of adenovirus 2 transactivates viral and cellular gene promoters through binding sites for TFIID and ATF (a factor that may be related to or identical to CREB) (*65*). The E1A transactivation function has been delimited to a 49–amino acid region present in the larger of two E1A proteins derived by differential splicing (*66*); but it is not known whether this domain directly contacts either of the above-mentioned DNA binding factors. The effects of herpes simplex virus trans-activator protein VP16 are also mediated through two different promoter cis elements. VP16 lacks a DNA binding domain, but has a COOH-terminal acidic region that is a potent transcriptional activation domain when linked to or associated with a DNA binding protein (*67*).

E1A can indirectly repress transcription of genes controlled by certain viral and cellular enhancer elements, and a number of studies indicate that repression is mediated by a region of E1A that is separate from the transcriptional activation domain (*68*). Another viral transforming protein, large T antigen of simian virus 40 (SV40), shares this ability to both activate and repress transcription. T antigen negatively regulates viral early transcription by at least two independent mechanisms. First, T antigen can sterically block initiation at the SV40 early promoter by binding directly to DNA sequences that overlap the early initiation site (*69*). Second, T antigen can form a complex with the cellular transcription factor AP-2 and thereby inhibit the specific binding of AP-2 to SV40 enhancer elements that are important for early transcription (*70*). As the latter mechanism does not involve direct binding to DNA, it is more likely to be related to the transcriptional repression mechanisms used by E1A.

Future Prospects

Studies of DNA binding transcription factors in animal cells have led to the discovery that they are a remarkably diverse group and have been constructed from a variety of combinatorially arranged functional domains. The mechanisms by which myriad sequence-specific DNA binding factors affect different rate-limiting steps in transcription initiation are largely unknown, and future experiments will be aimed at understanding, at the biochemical level, how different transcription factors activate initiation and which proteins they interact with in order to accomplish this. A more complete understanding of the initiation process will require new, innovative approaches directed toward the dissection of intact macromolecular initiation complexes. Also, studies that connect cellular signal transduction systems to the regulation of transcription factor activities will be particularly important for our understanding of cellular growth, differentiation, development, and oncogenesis.

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71. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

72. We thank the many colleagues who contributed preprints and those who critically read this manuscript and made suggestions for its improvement, especially D. Baltimore, H. A. Barker, L. Chasin, H. Echols, M. Koshland, and S. McKnight. We thank K. Ronan for help in preparing the manuscript, and the members of the Tjian laboratory, in particular M. Biggin, D. Bohmann, B. Lüscher, and R. Turner for helpful discussions. P.J.M. is supported by a Damon Runyon–Walter Winchell Cancer Fund Fellowship, DRG-892.