TRANSCRIPTION ELEMENTS AND FACTORS OF RNA POLYMERASE B PROMOTERS OF HIGHER EUKARYOTES

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I. GENERAL INTRODUCTION

Genetic information is expressed in a sufficiently controlled way so that organisms develop, reproduce, and pass on the information to future generations. In higher eukaryotes, there are many steps at which gene expression is controlled, including (1) the accessibility of genes to transcription factors in exposed as opposed to condensed inactive chromatin;[1-4] (2) the rate of specific initiation of transcription on open chromatin;[5-11] and (3) various post-transcriptional steps.[12-16] The rate of RNA chain initiation is an important point at which gene expression is controlled. Eukaryotic genes can be classified according to the type of RNA polymerase by which they are transcribed. The three known types of RNA polymerase — A, B, and C (also called I, II, and III)[17-19] — transcribe ribosomal RNA genes, protein-coding and small nuclear U RNA genes, and 5S and tRNA genes, respectively. This review concerns the protein-coding genes of higher eukaryotes, which are the most heterogenous class of genes, and which are especially important because they code for the regulatory proteins, e.g., those that control development.[20,21] I will deal with the trans-acting factors which regulate both the amount and site of specific RNA chain initiation and the DNA sequences, or promoter sequences, with which they interact.

II. TECHNIQUES

The great advance in recent years in our understanding of eukaryotic promoter structure results mainly from two technical breakthroughs: (1) mutagenesis or the ability to isolate and mutate specific DNA sequences and (2) development of methods in vivo and in vitro to study phenotypic expression of the in vitro-generated mutants. Several of these techniques are discussed because they influence and limit our understanding of promoter structure and regulation.

A. Mutagenesis

Recent advances in DNA manipulation make the systematic mutagenesis, nucleotide by nucleotide, of up to 300 base pairs (bp) of DNA, within the reach of most laboratories.[22-25] Hence, every nucleotide of a promoter can be tested for its role in transcription, without any restriction such as in vivo phenotype. There are several recent reports of systematic

genetic analysis of polymerase B promoter elements.[26,27] For the simian virus 40 (SV40) enhancer,[26] a combination of rapid small-scale synthesis of oligonucleotides with microscale "shotgun gene synthesis" was used for directed mutagenesis. The segmented microscale paper method allows the synthesis of about 100 oligonucleotides 20 bases long in 3 days.[28] These oligonucleotides can be ligated into an expression vector to efficiently synthesize up to 300 bp of DNA of any desired sequence. By changing two complementary oligonucleotides, mutations can be introduced into any part of the reassembled DNA (see Figure 1A). One hundred 20-mers are sufficient to mutate every nucleotide of a 50-bp DNA fragment. In the second report, for the β-globin promoter, a technique was used that allows DNA molecules of 30 to 600 bp, differing in sequence by 1 bp, to be separated on a preparative denaturing gradient gel (see Figure 1B).[29,30] Single-stranded DNA is randomly modified with mutagens; the region of interest is made double stranded by primer extension, and then is inserted into a vector containing a special "GC clamp". The target DNA + GC clamp is fractionated on a denaturing gradient gel to separate mutated DNA from the excess of wild-type DNA which remains due to the limiting mutagenesis conditions required to generate single-base mutations. Up to 95% of all possible single-base substitutions are obtained by this method. The first method has the advantage that the types and number of mutations can be chosen at will.

B. In Vivo and In Vitro Assays

A variety of techniques exists to study both the effects of promoter mutations on initiation of transcription and the trans-acting factors which interact with promoter sequences. These can be classified as in vivo or in vitro techniques, depending upon whether live animals and cultured cells or acellular extracts are used (for bibliography, see References 31 to 38).

1. In Vivo Assays

a. Expression of Transfected DNA in Cell Lines

The most common approach in permanent cell lines in culture is the transient transfection assay. DNA is applied to cells with chemicals which facilitate both entry into the cell (calcium phosphate precipitates, DEAE dextran, etc.) and subsequent expression (chloroquine, dimethylsulfoxide, glycerol, etc.). Gene expression is maximal after about 48 hr, and generally disappears after around 80 hr. Although the technique is very useful, there are many limitations:

1. Only a small number of cells take up DNA, and only a small proportion of the DNA which reaches the nucleus is transcriptionally active.[39,40] This complicates analysis of the transcriptionally active DNA for methylation, associated proteins, topology, etc. Although a fraction of the transfected DNA is organized into a regular chromatin structure[41,42] and replication increases this fraction,[378] the structure of the transcriptionally active DNA is not precisely known.
2. The exogenous DNA is expressed episomally and outside the normal chromosomal context. This may account for the unusual expression of some transfected genes. Certain promoter sequences are inactive when the transfected DNA is episomal, but are active when the DNA is stably inserted at any one of a number of sites in the chromosome (e.g., see Reference 43). An exogenous β-globin gene transfected into fibroblasts is transcribed about 10^5 times better than the endogenous gene. This is still about 10^3 times less than in a red cell.[44-46] The normal chromosomal context may determine the reduced rate of transcription of the endogenous gene.
3. There is an appreciable rate of modification of transfected DNA: 15 to 20% of transfected DNA may be mutated, and about 1 double-stranded break generated per 5,000 to 15,000 bp. Linear DNA is rapidly ligated together into high molecular weight

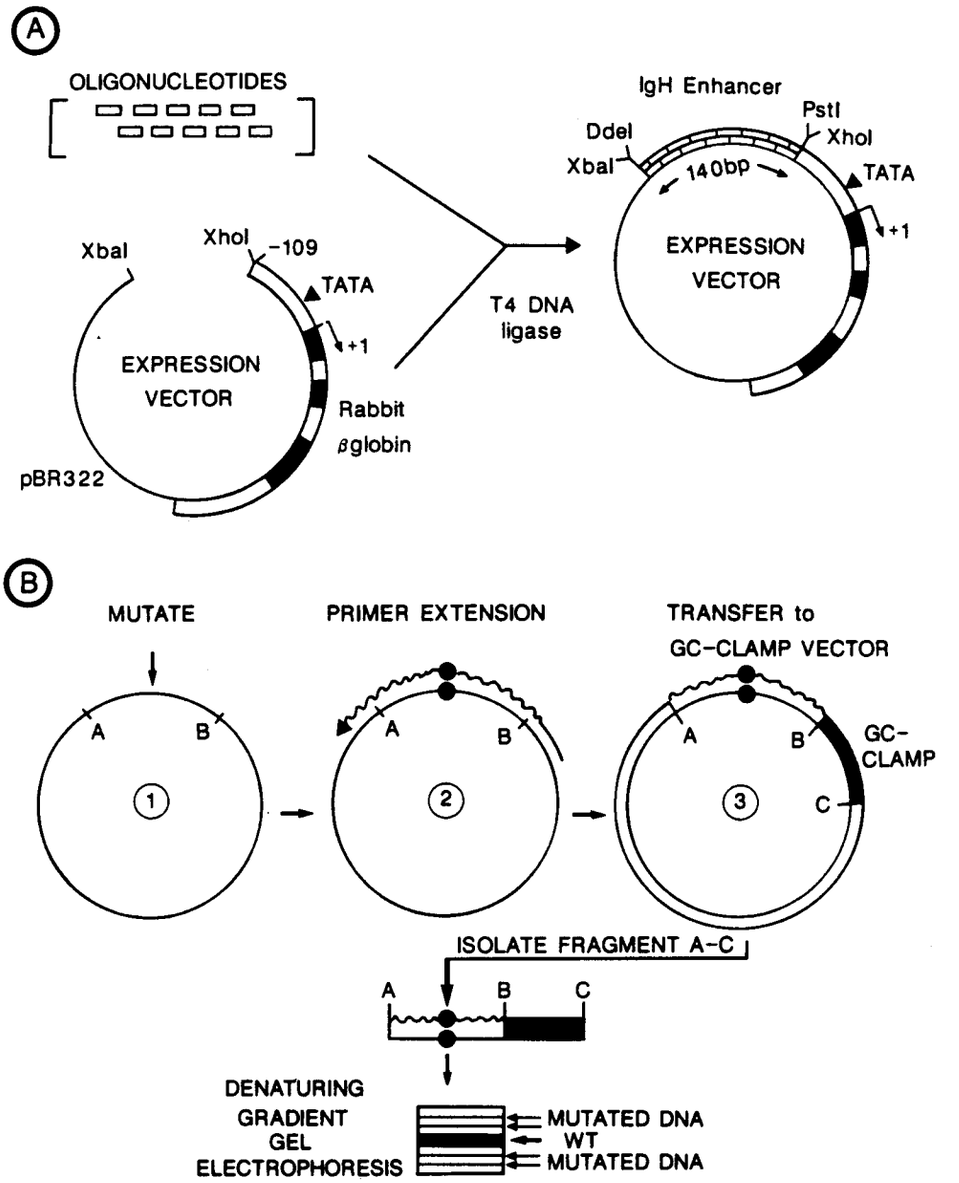


FIGURE 1. (A) Direct synthesis of immunoglobulin heavy-chain enhancer fragment in expression vector. Oligonucleotides synthesized by the segmented paper method are ligated directly into the expression vector for transfection into mouse cells. Up to 90% of the synthesized fragments of about 150 bp are of the expected sequence if the oligonucleotides are extensively purified, and equivalent amounts are ligated together[384] (see Section II.A). (B) Mutagenesis procedure. The DNA of interest in a single-stranded form, and surrounded by unique restriction site A and B (1) is mutagenized, made double stranded (2), and the fragment of interest, A-B, is recloned into a GC-clamp-containing vector (3). After transformation of (3) in *E. coli*, pooled colony DNA is fractionated on a denaturing-gradient gel which separates mutated DNA fragments from the wild-type (WT) DNA (see Section II.A). The dots between A and B represent the mutated nucleotides, and the wavy line represents the newly synthesized DNA.

oligomers.[47-54] However, this level of modification should not influence most studies of promoter function.

4. The facilitating agents are not effective for all cell lines, and a variety of more sophisticated techniques can be used (for bibliography, see References 31 to 38).

- Cell fusion. Cells are fused with either bacterial protoplasts or artificial membrane vesicles (liposomes) containing the test DNA.
- Microinjection. The DNA is microinjected directly into the cytoplasm or nucleus. This technique requires specialized equipment, and only a small number of cells can be injected (500 to 1000/hr). However, the efficiency of transfer and expression is high (50 to 100%) and it is particularly useful when only a small number of cells (e.g., fertilized mouse eggs) or a small amount of DNA are available.
- Electroporation. Cells are exposed to an intense electric field (1 to 20 kV/cm) for a short period of time (1 to 5 μsec). Transient pores form in the membrane which allow DNA in the surrounding solution to enter the cell.
- Viral vectors. The efficient viral pathways for cell infection are exploited by integrating the test DNA into the viral genome and packaging it in the viral capsid. The study of the properties of the integrated DNA is facilitated by both the high proportion of viral genomes in the cell which are transcriptionally active, and template amplifications through viral replication. Particular properties of different types of virus are exploited, such as the size (180,000 bp) and extended host range of vaccinia virus, the short infectious cycle (24 hr) of adenoviruses, the capacity of retrovirus to stably integrate into the genome without killing the host, and the stable episomal state of bovine papilloma virus. Viral vectors have been used successfully to study the expression of several cellular promoters. The vectors were based on adeno-[55,56] and retroviruses.[57,58] However, viral sequences can influence expression of an internal promoter. An interesting example is promoter suppression in the retrovirus, spleen necrosis virus, where expression from an internal promoter is suppressed when the viral LTR promoter is active, and conversely, the viral promoter is suppressed when the internal promoter is active.[59]

Transfection of DNA into cultured differentiated cell lines has been used to study control mechanisms such as cell-specific regulation of transcription (examples include erythroid cells, lens, liver, lymphocytes, and pancreas[46,60-63]). However, many control mechanisms are not represented in permanent cell lines in culture and, even when appropriate cell lines exist, all the regulation exerted on the endogenous gene may not be reproduced on introduced DNA. For example, liver cells in primary culture rapidly lose transcription factors for the albumin gene, probably because of the disruption of cell-cell contacts.[64-66] β-Globin genes introduced into MEL mouse erythroleukemia cells are correctly regulated when the cells are induced to differentiate. However, the amount of β-globin mRNA transcribed per gene is 10- to 100-fold less than that from the endogenous gene. In contrast, the transfected α-globin gene is not correctly regulated during differentiation. Some of these difficulties might be overcome by introducing DNA either directly into tissues of adult animals (for bibliography, see Reference 67) or into the germline, to produce transgenic animals.[68-71]

b. Transgenic Animals

The most widely used technique for the production of transgenic animals is the microinjection of DNA into the pronucleus of fertilized mouse eggs. These eggs are implanted into pseudopregnant foster mothers. Newborn pups are analyzed for retention of exogenous DNA, and tissue biopsies are used to analyze for tissue-specific expression. The advantages of the method are that gene expression can be followed in every cell of the body in its normal

surroundings and throughout development. Transgenes are subject to control mechanisms which cannot be reproduced in cell culture, such as in the immune system, in the nervous system, or during growth. This can be illustrated with erythropoiesis, where the cell lines in culture simulate the in vivo pathway only in part. In transgenic mice, globin genes, as well as various other genes, show the same pattern of regulation as their endogenous counterparts.[73-82] The human β-globin gene is expressed like an adult mouse globin gene,[73,74] while the human fetal γ-globin gene is expressed like an embryonic mouse globin gene. Interestingly, a hybrid γβ-globin gene, with a combination of regulatory sequences, has an altered pattern of development expression, and is active at all stages in murine erythroid tissues.[83]

However, difficulties remain with the transgenic mouse technique. Even in the best hands, only 10% of injected eggs give rise to pups, and 25% of these contain an integrated gene.[69,71] This, coupled with the generation time of mice, limits the number of mutated genes which can be studied. The exogenous genes integrate in multiple copies, and since any one of these may be active, it is difficult to study the effects of chromatin structure, methylation, etc. on gene expression. Insertion can occur at any one of a number of locations, and the influence of the surrounding sequences may account for the transgenic mice which either do not express the foreign genes (30 to 50%), or express them to an extremely variable extent. This position effect suggests that there is a level of control which is not properly exerted, and that some regulatory sequences may be absent from the newly introduced genes.

It would be invaluable to be able to precisely replace or manipulate cellular genes by homologous recombination, as is standard practice in yeast.[84] There are several recent examples of gene targeting whereby introduced DNA has been shown to recombine with homologous sequences in the genome.[85-87] However, this is still very inefficient: homologous recombination is about 100 to 1000 times less efficient than nonhomologous recombination. There are naturally occurring mutations such as β thalassemias, but the effects of many natural globin mutations have not yet been assayed in transfection studies.[88] Discrepancies between the regulation of endogenous genes and the transfected DNA may provide insights into molecular mechanisms.

c. Measurement of the Rate of Specific Initiation of Transcription

Once the mutant promoter has been introduced and expressed, the next step is to assess promoter mutant phenotype. The important parameter is the change in rate of specific initiation, which is difficult to measure directly in vivo. The most direct approach has been to pulse-label cells and to measure RNA synthesized close to the RNA startsite. However, this is possible only for viral genes that exist in multiple copies.[89] The next best approach is the run-on transcription assay. Purified nuclei are incubated with labeled precursors of RNA to allow RNA polymerases engaged in transcription to synthesize short pieces of labeled RNA, which are quantitated by hybridization to specific DNA immobilized on a filter. Since each RNA polymerase probably synthesizes a similar length of RNA, the amount of hybridized labeled RNA is proportional to the number of RNA polymerases on the probe DNA.[90] Another indirect approach is to quantitate stable specific RNA which has accumulated during the transfection. Misleading results may be obtained if there are changes in RNA stability, or if there is attenuation of transcription. Many studies use indirect assays based on production of a detectable protein or enzyme from a "reporter" gene located directly downstream from the promoter. The assumption is that the amount of reporter product is proportional to the rate of specific initiation of transcription, and it is usually necessary to verify this supposition with one of the techniques described above. The advantages of the indirect approach are rapidity and sensitivity. A recently introduced reporter system is light production by the luciferase gene, which is thought to be 100 times more sensitive than current methods,[91,92] and should extend considerably the range of cells in which gene expression can be studied.

d. Detection of Trans-Acting Factors

A variety of techniques exists to detect proteins associated with promoter sequences in vivo. The most elegant approach is genomic footprinting, in which cells are incubated with dimethyl sulfate (DMS), which modifies G residues in DNA. The modified DNA is extracted, cleaved at the methylated bases, and the site of modification determined with single-nucleotide resolution by genomic sequencing.[93] Differences in the extent of G modification between different types of cells, or after induction of transcription, reflect changes in the environment of the bases, possibly due to the proteins interacting with the DNA. This technique has been applied in only a limited number of cases,[94,95] most probably due to technical difficulties associated with genomic sequencing. A less direct approach is to use isolated nuclei, which permit more DNA modification reagents to be used, such as endo- and exonucleases and chemicals which cleave DNA. Again, the site of cleavage can be determined with single-nucleotide resolution by genomic sequencing[96,97] but, most commonly, less resolutive techniques are used (for reviews, see References 1 to 3).

2. In Vitro Assays

In vitro transcription systems which accurately reproduce transcriptional controls are important for deciphering the precise molecular mechanisms underlying control of gene expression. The significant breakthrough came less than 10 years ago when Weil et al.[98] and Manley et al.[99] prepared extracts from HeLa cells in which RNA polymerase B initiated transcription specifically (for reviews, see References 100 and 101). In vitro extracts reproduce some of the transcriptional controls exerted on cellular and viral promoters. For the SV40 early promoter, they reproduce many of the transcriptional effects of promoter mutations observed in vivo,[102-104] as well as repression by viral large T antigen.[105] Cell-specific in vitro transcription has been demonstrated in extracts from various cell lines (red blood cell and B lymphocytes),[106-109] and from tissues (rat liver and silk glands).[110,111] Other transcription regulatory functions that have been reproduced are enhanced transcription in response to viral trans-activating factors,[112,113] cell-cycle regulation,[114] and heat-shock induction.[115]

The in vitro extracts are being used to purify the factors involved in regulation of transcription. Several recently introduced techniques have had an enormous impact on the detection and purification of transcription factors. Originally, specific initiation of transcription was measured by the run-off assay, in which RNA polymerase transcribes a truncated template containing a promoter. RNA initiated at the specific startsite and extending to the end of the truncated template has a defined size, and can be separated from nonspecific RNA by gel electrophoresis (see Figure 2A). Specific transcription is quantitated by measuring radioactivity in the specific size-labeled RNA band. Three techniques have superseded this laborious method to assay for the presence of transcription factors. Sawadogo and Roeder[116] designed an artificial run-off template lacking G residues downstream from the promoter. In the absence of GTP, RNA polymerase can only synthesize RNA on the artificial run-off template. Since the majority of this RNA is specifically initiated RNA, then specific transcription can be quantitated simply by measuring total RNA synthesis. The other methods detect proteins which bind specifically to promoter sequences. In the footprinting assay (see Figure 2B), end-labeled DNA is incubated with extract and then partially digested with DNAse I. A sequence-specific binding protein will modify DNAse I cutting at the site of interaction relative to naked DNA, leaving an imprint that can be detected by gel electrophoresis.[117] A variety of other modifying agents can be used in the place of DNAse I, such as exonuclease III and DMS.[1-3] In the gel-retardation assay (see Figure 2C), the specific protein-DNA complex is detected by its decreased mobility relative to uncomplexed DNA on polyacrylamide gels.[118-120] Both footprinting and gel retardation are very sensitive due to the high specific activity of DNA probes, thereby permitting the detection of tiny amounts

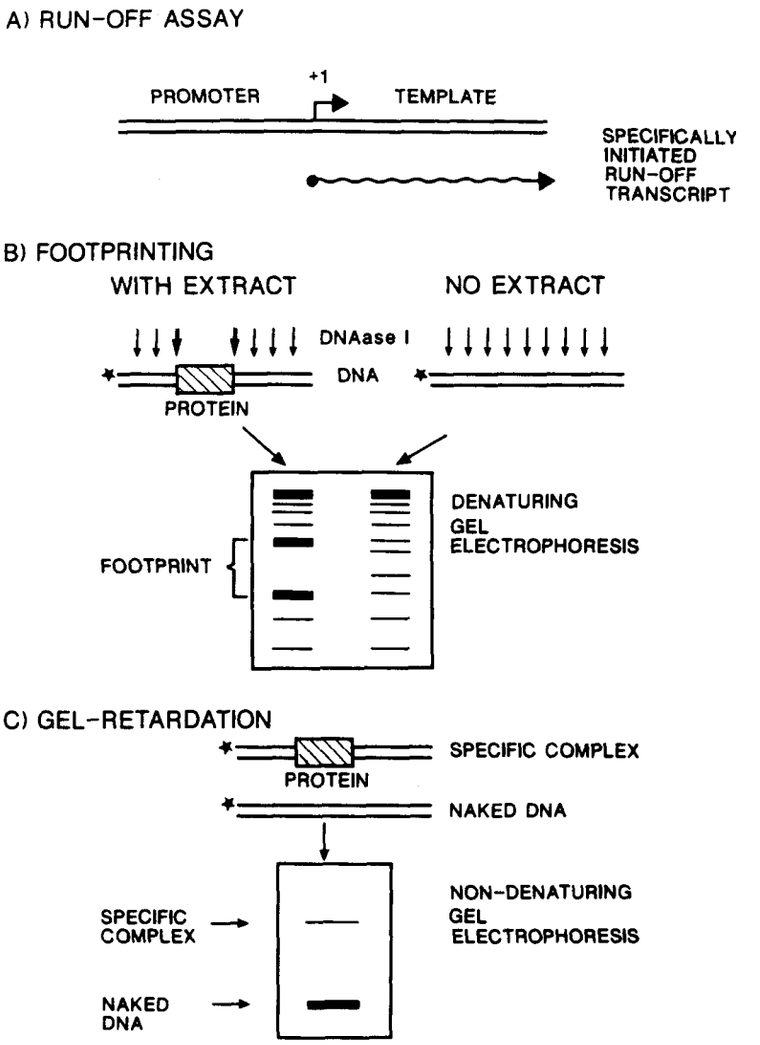


FIGURE 2. Methods for detecting in in vitro extracts specifically initiated transcripts (A) or sequence-specific DNA binding factors (B, C). In the run-off assay (A), a promoter containing DNA fragment is incubated with cell extract and radioactive RNA precursors (XTP). Specifically initiated RNA which extends to the end of the template has a known size and can be detected by gel electrophoresis. In footprinting assays (B), proteins specifically bound to DNA alter its reactivity to various reagents, such as DNAse I. The binding site is localized by gel electrophoresis of the radioactive end-labeled (*) DNA. Stable protein-radioactive-DNA complexes can also be identified by their decreased mobility compared to naked DNA on nondenaturing gel electrophoresis (gel retardation, C).

of material. However, additional information, from in vivo assays, is required to establish the functional importance of a sequence-specific DNA binding factor. High-affinity sequence-specific protein-DNA interactions have also been exploited for affinity chromatography, which has recently permitted complete purification of several rare DNA binding proteins such as Sp1[121] and MLTF.[122] In one technique, multimers of chemically synthesized oligonucleotides, which contain a recognition site for a sequence-specific DNA binding protein, are linked to a Sepharose column and used for affinity chromatography. The Sp1 transcription factor was enriched 500- to 1000-fold by this technique.[123] In the second technique, a biotin

UTP end-labeled DNA fragment containing a recognition site is complexed with the sequence-specific binding protein in solution. The nucleoprotein complexes are then bound to streptavidin-agarose and used for affinity chromatography. This technique gave a 250-fold purification of the major late transcription factor (MLTF).[122] Cell-specific gene expression appears to be mediated by factors that interact specifically with DNA (e.g., SV40, insulin, and the immunoglobulin heavy- and light-chain genes[124-130]). The exciting prospect is that many of these factors will soon be purified both to allow a study of their role in transcription and, through classical molecular biological routes, to clone the corresponding genes. It is possible that by studying control of expression of these genes, we will learn about how differentiation is controlled, and thereby perhaps identify hierarchical control mechanisms analogous to those involved in *Drosophila* development.[131]

III. TRANSCRIPTION IN PROKARYOTES

A. General Principles

The sequences which govern specific RNA chain initiation in prokaryotes have been divided into promoter sequences, which are specific binding sites for RNA polymerase, and regulatory sequences, which bind regulatory molecules that modulate the rate of RNA chain initiation. The major form of RNA polymerase consists of a core enzyme, and a dissociable σ subunit which specifies the class of promoter sequence recognized (for reviews, see References 132 to 135). The predominant form of RNA polymerase in *Escherichia coli* contains σ^{70} and binds to promoters whose consensus sequence is illustrated in Figure 3A. The consensus sequences is derived from over 100 known promoter sequences and is the best promoter sequence for this enzyme. Modifications that alter the sequences around $+1$, -10 (Pribnow-Schaller box), or -35, or their spacial relationship to one another, decrease promoter strength. The basal rate of initiation of transcription from a promoter is determined by its sequence, and repressors and activators serve to alter this basal rate.

Classically repressors bind to sequences within or close to the promoter (Figure 3B) and sterically prevent RNA polymerase-promoter binding. However, steric factors cannot always account for repression. In the galactose operon, two operators are required for inhibition: one at -60 and the other at $+50$. Neither is in a position to directly inhibit RNA polymerase. Perhaps the conformation of the DNA between the operators may inhibit access of RNA polymerase to the promoter. Activators bind just upstream from the -35 region (Figure 3C) and, by protein-protein interactions, either increase polymerase-promoter binding or the rate of isomerization of bound polymerase from a closed complex to an active open complex which can rapidly initiate RNA synthesis. However, direct interprotein contacts cannot explain all types of activation. For example, the catabolite gene activator protein (CAP) binding sites are found at many different locations with respect to the promoter. CAP protein has a large binding site (about 30 bp) compared to other regulatory factors, and induces a bend of over 90° in the DNA. This may store energy which can be used to facilitate transcription.[136]

B. Looping

In the compact prokaryotic genome, most control sequences are clustered around the RNA startsite. However, there are control sequences which can affect transcription even when they are located at considerable distances from the promoter. There is a growing number of both repressors and activators which can regulate transcription at a distance, and the mechanism of this distal regulation has important parallels in higher eukaryotes. In the araBAD operon, there is a repressor binding site close to the promoter and an additional site about 200 bp farther upstream which is required for complete repression.[137] In the deo operon, full repression by a promoter proximal repressor requires an additional repressor binding

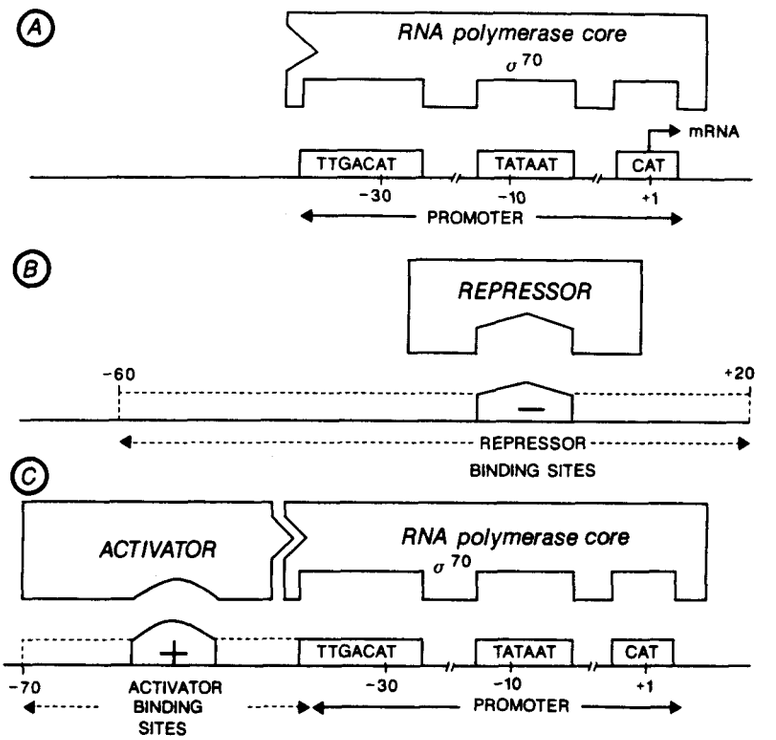


FIGURE 3. Transcription initiation in prokaryotes. The RNA polymerase core-σ^{70} holoenzyme interacts with the promoter principally through the sequences around +1, −10, and −35 (A). The classical mechanism of repression (B) is by steric hindrance of RNA polymerase-promoter interactions by repressors bound to the region extending approximately from −60 to +20, while the classical mechanism of activation involves activator binding to proximal upstream binding sites and protein-protein interactions with RNA polymerase which stimulate its activity.

site about 600 bp away.[138] In the lactose operon, with an operator constitutive mutation which decreases repressor binding to the promoter proximal site, insertion of wild-type operator sequences hundreds of base pairs upstream or downstream from the promoter aids repression.[139,140] In the glnA promoter region, there are 5 activator sites upstream from the promoter, the 2 strongest lying upstream from the RNA startsite around −110 and −140 bp. Moving all but a weak promoter proximal site to over 1000 bp upstream did not diminish the ability of the activator to stimulate transcription, suggesting that activators bound to the distantly located operators interact with the proximal activator which in turn stimulates RNA polymerase by protein-protein interactions.[141] It seems likely that the presence of additional activator or repressor binding sites increases the local concentration of regulatory molecules in the vicinity of the promoter. This could be accomplished either by sliding of repressors on the DNA, or by intersegmental transfer from one operator site to another.[142] For the latter to occur, a loop has to form, either transiently, or as a permanent structure. A loop held together by repressor molecules can be expected to be stable because of the low probability of dissociation from both sites at the same time. Dissociation from one site would be reversed by the repressor at the other site.

There is evidence for loop formation. In the araBAD operon, small deletions or insertions which result in alterations in the distance between the two repressor binding sites of half-integral numbers of helical turns decrease repression, but alterations of integral numbers of

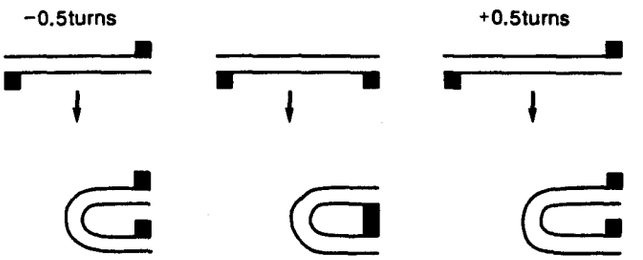


FIGURE 4. Protein-protein interactions and looping. Proteins (■) can interact when they are not adjacent to each other on the DNA by looping out of intervening DNA sequences. Proteins located on the same face of the helix interact more easily than those located on opposite faces of the helix (DNA with +0.5 or −0.5 turns) because of the additional twisting of the DNA required to bring them together.

turns do not.[143] A reasonable explanation is that proteins separated by several hundred base pairs can interact through looping only if they are placed on the same side of the helix (see Figure 4). Changes in spacing of half-integral helical turns place them on opposite sides of the helix, so that the energy required to twist the DNA inhibits loop formation. Loop formation can be expected to be sensitive to the distance between the ends of the loop because of the rigidity of the DNA helix. It should become increasingly more difficult to bring two sites on the DNA together by looping as the chain length between them decreases below about 250 bp. In agreement with these predictions, it has been shown that upstream lactose operators separated from the promoter proximal mutated operator by over 200 bp are more effective in repression than when separated by about 120 bp.[139] Loop structures can be seen by electron microscopy when λ repressors are bound to λ operators separated by five helical turns of DNA. Loops do not form when the separation is 4.5 or 5.5 helical turns.[144] Interestingly, the DNA helix in small loops of about five helical turns is significantly compressed on the inner surface of the loop, and expanded on the outside of the loop.[145] This altered topology could stimulate or inhibit protein binding to the looped DNA, and conversely, proteins bound to the intervening DNA could prevent loop formation and repressor interactions.

IV. TRANSCRIPTION IN HIGHER EUKARYOTES

A. Introduction

I will distinguish two types of promoter sequences in protein coding genes which are transcribed by RNA polymerase B: those which select the site of initiation (selector sequences; see Sections IV.B and IV.D.1) and those which modulate the rate of specific initiation (upstream elements and enhancers; see Sections IV.C to F). The selector region extends from about −45 to +30, and contains 2 highly conserved motifs, the TATAAA sequence around −30 and CA at +1 (see Figure 5). General transcription factors bind to this region and direct RNA polymerase B to the specific startsite. The same general factors are probably required for specific initiation from most protein-coding genes. The selector region and the prokaryotic promoter (the −35 to +10 region) are similar in that both are specific binding sites for RNA polymerase in the presence of auxilliary factors, and both are sufficient for a basal rate of initiation of transcription in the absence of other specific DNA sequences and factors, particularly in vitro. However, the prokaryotic promoter is sufficient for strong specific transcription in vivo, whereas it appears unlikely that the same is true for the selector in higher eukaryotes.

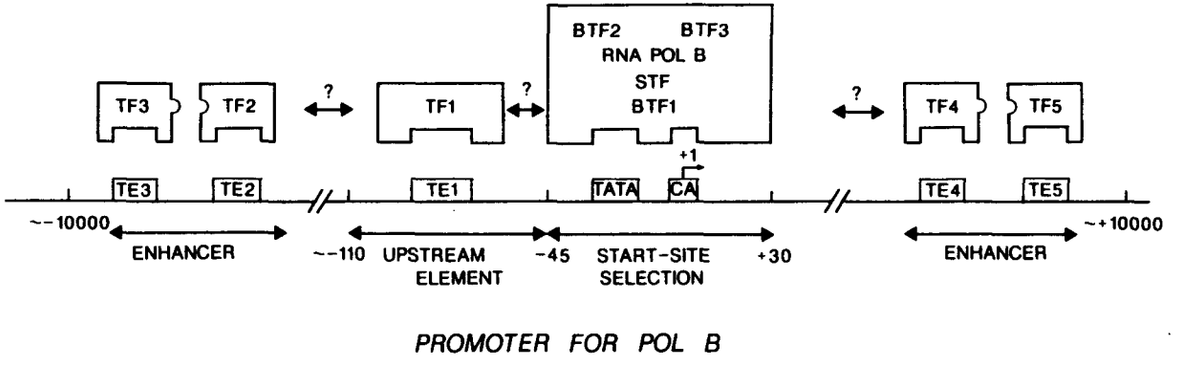


FIGURE 5. Model of a eukaryotic protein-coding gene promoter. The startsite selection region interacts with general transcription factors which are common to many RNA polymerase B (RNA pol.B) promoters. The sequence-specific startsite selection region DNA binding protein is BTF1 (also called TFIID), possibly in association with STF (TFIIA). This protein-promoter complex is recognized by RNA pol.B, possibly in association with two other factors (BTF2, BRF3, or TFIIE, TFIIB). Various other transcription factors (TFs) bind to specific sequences or transcription elements (TEs) which can be located at least up to 10,000 bp upstream or downstream from the RNA startsite (+1). It is common to find at least 1 TE in the region immediately upstream from the startsite selection region (from −45 to −110, upstream element), although it is not definitely established that there is a distinct subclass of TEs (and corresponding TFs) associated with this region. Efficient distal activation of transcription by enhancers appears to require the association of several TFs. The types of interaction which may exist to generate an active promoter are presented either by double-headed arrows with question marks or by complementary shapes of the symbols. Transcription factors which do not bind to DNA may be required for these interactions.

In most protein-coding genes, there are sequences up to about 100 bp upstream from the RNA startsite which regulate transcription from the RNA startsite (see upstream element, Figure 5). This region contains one or several short sequences, of about 10 to 20 bp, or transcription elements (TEs), which serve as recognition sites for transcription factors (TFs) (see SV40 early promoter 21-bp repeats, Section IV.D.2). This can be considered to be the basic organization for genes which either are expressed at a similar level in most cells or respond in a simple fashion to one or several control events (e.g., *Drosophila* HSP70 heat-shock promoter, see Sections IV.B and IV.C). More complex regulation is achieved by additional sequence elements which can be located and can function at distances up to at least 10,000 bp upstream or downstream from the RNA startsite (see enhancer, Figure 5). A special term, enhancer, has been coined for any promoter sequence that can stimulate transcription in an orientation-independent fashion when located more than several hundred base pairs upstream or downstream from a promoter. However, it appears that many TFs are capable of both short- and long-distance activation, and that there may not be specific upstream or enhancer factors (see Section IV.C). Distal elements appear to contain at least two TEs which interact with the corresponding specific TFs. Synergistic interaction between multiple TE-TF complexes in the distal elements is probably required to generate promoter elements which are sufficiently active to stimulate promoter activity at these distances. The mechanisms of synergistic interaction are not understood at present (for an illustration of synergistic and nonsynergistic interactions, see SV40 enhancer, Section IV.D.3.b). Promoters can be built up from a large number of different TEs and TFs. TFs can vary in activity either in response to a variety of cellular signals (hormones, heat, etc.) or from cell type to cell type. Control of promoter activity will depend upon both the number and the type of TEs which it contains. This combinational organization of the promoter could lead to quite complex regulatory patterns with only a small number of TFs. How particular combinations of TFs lead to given patterns of regulation is only beginning to be understood. Several extensively studied promoters are described in detail to illustrate how a relatively nonspecific promoter element can be generated from TEs which interact with both specific and ubiquitous TFs (SV40 enhancer, Section IV.D.3), how both specific and ubiquitous positive as well as negative factors generate a tissue-specific enhancer (IgH enhancer, Section IV.E), and how variation of TF activity controls gene expression during differentiation (Igκ enhancer, Section IV.F). The combinatorial possibilities may be limited by the inability of certain factors to interact with each other. This may explain why certain combinations of promoter elements work better than others (see SV40 enhancer, Section IV.D.3.d), and how a combination of tissue-specific and ubiquitous dc-octamer elements can activate transcription nonspecifically from some promoters and tissue specifically from others (see Section IV.E).

The in vivo and in vitro techniques that are used extensively (Section II.B) lead to a model of the promoter in which a variety of TFs are bound specifically to the DNA, and somehow interact with each other, possibly by protein-protein interactions, to generate a multifactor-DNA complex. If the factors are distantly located, then looping out of intervening DNA allows protein-protein interactions (see discussion of looping in Section III.B). Most of the evidence for this type of organization comes from extensive studies on the SV40 promoter (Section IV.D.3.e). This model of the promoter-factor complex does not explain why certain promoter elements are apparently dispensable after transcription has been switched on (Section IV.D.3.f). In addition, various other components are involved in transcription regulation, and again SV40 early promoter is used to illustrate how chromatin structure (Section IV.D.4), matrix attachment (Section IV.D.5), torsional stress (Section IV.D.6), and DNA structure (Section IV.D.7) may affect transcription. Finally, transcription and replication are tightly coupled, especially in the SV40, polyoma, and adenoviruses. Surprisingly, in several cases, the same factors are responsible for regulation of both transcription and replication (Section IV.D.8).

B. Startsite Selection and Basal-Rate Transcription

1. A Large Multicontact Interaction Site for General TFs

Early studies of the promoter sequences of a small set of RNA polymerase B (pol.B) promoters revealed two regions of sequence homology: the −30 TA motif (TATA box)[146-148] and the −80 CAAT box.[149,150] The analysis of a larger set of promoters, together with functional analysis, has shown that the most generally conserved sequences lie in the −45 to +30 region, and that particular upstream homologies are restricted to various size subsets of pol.B promoters (see below). A recent analysis of 168 eukaryotic promoters shows that the conserved features of the −45 to +30 region are the 5′-TATAA-3′ sequence centered around −28, a bias towards G-C base pairs around the TA motif, and the 5′-CA-3′ sequence with A at +1.[151] A surprising result is that the 5′-TATAAA-3′ sequence is as highly conserved as the *E. coli* Pribnow-Schaller box, even though the eukaryotic sequences are derived from a wide spectrum of sources.

Function studies, both in vivo and in vitro, suggest that the sequences in the −45 to +30 region form a binding region for general transcription factors that are common to most if not all pol.B promoters. The two conserved sequence motifs are the primary binding sites, although other sequences are most probably also required for the interaction. The primary interaction sites do not necessarily contribute equally to binding, and in certain cases sequences in and around the TA motif may predominate, while in others sequences around the startsite may be more important. Promoters without a consensus TA motif probably belong to the second category. The consensus sequences are the best promoter sequences, and the closer the fit to the consensus, the better the promoter. These conclusions are supported by the following observations.

Footprinting experiments, both in vivo[152-154] and with crude extracts or purified factors,[155-159] have shown that the −45 to +30 region is protected by TFs from exonuclease III or DNAase I digestion. Point mutations in the TA motif or 5′-CA-3′ sequence which decrease the fit to the consensus decrease both the rate of transcription in vivo and in vitro[27,160,161] and the factor binding in vitro,[159,162] while mutations which increase the fit have the opposite effect. The latter is illustrated by the SV40 late promoter. The −31 to −21 region has the sequence 5′-GGTACCTAACC-3′, and point mutations which increase the fit increase both the rate of transcription from the major cap site in vivo[379] and in vitro[163] and the affinity for general transcription factors.[159]

Two types of promoter can be distinguished: those in which both TATA and startsite regions contribute significantly to promoter strength and startsite selection, and those in which either region will have a predominant effect. The former situation is illustrated by the adenovirus major late promoter (ADMLP). Point mutations in either the TA motif or the startsite decrease transcription, whereas mutation of both sequences has a synergistic effect, eliminating transcription.[161] Transcription factors can bind to either the ADMLP TATA (−35 to −13) or startsite (−21 to +15) sequences independently, and either can compete for complex formation to the whole promoter.[159] However, either region can function independently, albeit at lower efficiency, since DNA fragments containing the TATA region and heterologous sequences in the place of the startsite direct transcription to start about 30 nucleotides downstream from the TA motif,[146] while the selector region in the absence of a TATA box will to some extent direct initiation of transcription to the normal startsite.[159,164] In the SV40 late promoter, which has no TATA homology sequence, the startsite regions are the primary determinants of the site of initiation. This is shown by the effects of small deletions or insertions upstream from the startsites, which do not alter the locations of the 5′ ends of the RNA upon infection with the mutant viral strains in vivo.[165] In the rainbow trout protamine TG3 gene, changing the spacing between the TA motif and the startsite by 3 bp leads to initiation at a fixed distance downstream from the TA motif,[166] suggesting that in this case the sequences in the region of the TA motif are the primary determinants of the

interaction leading to initiation of transcription. Besides the TA motif and startsite regions, other sequences may be involved in complex formation. In the silk-fibroin gene, mutations in the −20 region as well as in the −30 and +1 regions decrease transcription efficiency.[167] This suggests that there is one-sided contact between general general TFs and a promoter DNA with an approximately 10 bp per turn B-type helix.

Promoters with no apparent TATA homology, such as the ADE2a, AD2 IVa2, and SV40 late promoters, form complexes with the same purified TFs as other TATA-box-containing promoters; however, the affinity is lower for the nonconsensus promoters.[159] This observation helps explain various results. Mutation of one of the two TATA-like elements in the ADE2a promoter results in the more efficient use of the other one.[168,169] The AD2IVa2 and ADMLP promoters are separated by 210 bp, and the ADMLP promoter is transcribed more efficiently in vitro, probably because it has a consensus TATA sequence. A mutation in the ADMLP TA motif results in increased transcription of AD2IVa2.[170] In both cases, the results suggest that the promoters are in competition for a limited number of general TFs, and mutation of the more consensus-like sequence results in more efficient transcription from the other sequence.

2. The General TFs

Purification of TFs from crude extracts has shown that at least four factors and RNA polymerase are required for specific initiation from the selector region.[171-173] These factors have been given different names. The TFs, STF, BTF1, BTF2, and BTF3[171] are equivalent to TFIIA, TFIID, TFIIE, and TFIIB,[172,173] respectively, and AB, CD, and CB (CB ≡ BTF2 + 3 or TFIIE + B),[174] respectively. Using extensively purified fractions, it has been shown that two of these factors, STF (TFIIA) and BTF1 (TFIID), act first to form a stable preinitiation complex on the −45 to +30 region of the promoter[159,162,173,175] (see Figure 6) which commits the template to transcription. BTF1 (TFIID) alone can bind specifically to the −45 to +30 region of the ADMLP promoter, and with lower affinity to other nonconsensus TATA promoters.[159] STF (TFIIA) does not bind DNA specifically, but appears to stabilize the interaction between BTF1 (TFIID) and the promoter by protein-protein interactions.[175,176] Kinetically, STF (TFIIA) is required before BTF1 (TFIID) for complex formation and, since STF (TFIIA) may bind DNA nonspecifically, it has been suggested that STF (TFIIA) stabilizes or induces a DNA secondary structure required for BTF1 (TFIID)-DNA interactions.[173] STF (TFIIA) may be an actin-like molecule (e.g., see References 173, 176, and 177).

In a subsequent step in initiation of pol.B, BTF2 (TFIIE) and BTF3 (TFIIB) associate with the preinitiation complex to help form a complex which can rapidly initiate RNA synthesis (rapid start complex, Figure 6).[172,173,175] BTF2 (TFIIE) and BTF3 (TFIIB) do not bind specifically to DNA, and several observations suggest that they interact with other factors by protein-protein interactions. BTF3 (TFIIB) interacts with pol.B, as shown by cosedimentation studies on glycerol gradients[172,178] or by affinity to a column containing immobilized pol.B.[179] BTF2 (TFIIE) interacts with BTF3 (TFIIB) during cosedimentation on glycerol gradients.[172] Pol.B in the absence of BTF2 (TFIIE) and BTF3 (TFIIB) can associate with the preinitiation complex and increase its resistance to a low concentration of Sarkosyl (0.02%).[173] In Figure 6, these results are speculatively interpreted as BTF2 (TFIIE), BTF3 (TFIIB), and RNA polymerase interacting together to form a complex which can interact with a BTF1 (TFIID)-STF (TFIIA) complex bound to the selector region.

The multifractional-DNA rapid start complex forms in the absence of ATP. However, ATP hydrolysis at the $\beta - \gamma$ bond is required before formation of the first phosphodiester bond.[180,181] BTF2 (TFIIE) may catalyze this ATP hydrolysis.[180] There are at least two other discernible steps before a stable elongation complex is formed.[182,183] Specific factors stimulate RNA chain elongation.[184,185]

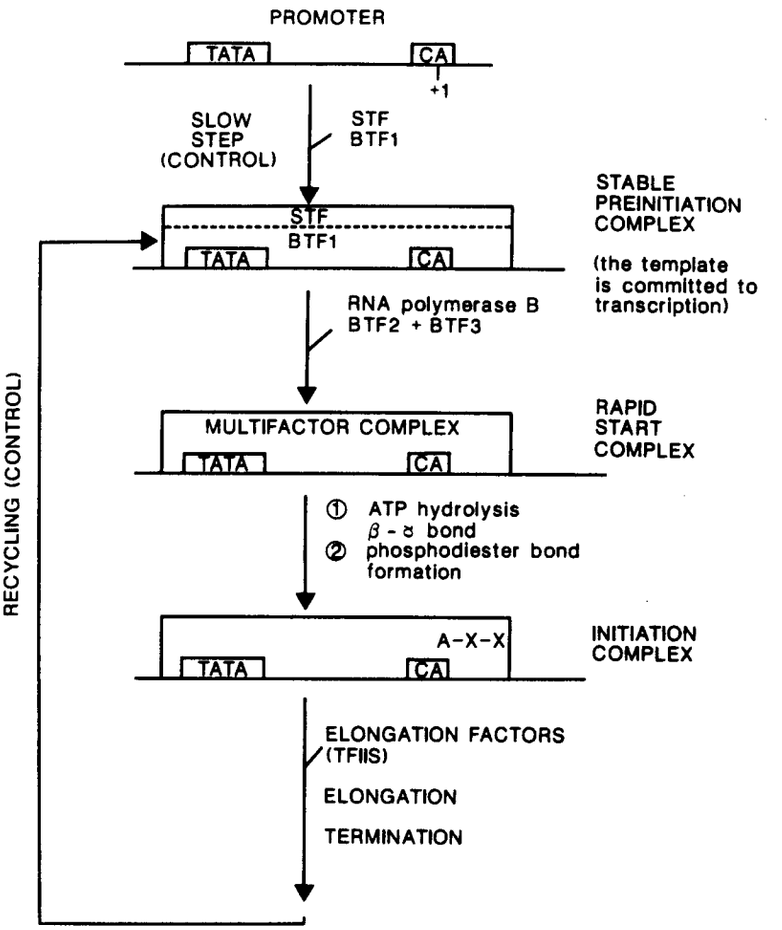


FIGURE 6. A hypothetical model for the minimal transcription initiation reaction from the startsite selection region. The first step involves association between the promoter sequences between −45 and +35 and general TFs STF (TFIIA) and BTF1 (TFIID) in a slow step to form a stable preinitiation complex. RNA pol.B, BTF2 (TFIIE), and BTF3 (TFIIB) associate with this complex to form a multifactor complex with the promoter which can rapidly initiate transcription when presented with nucleotide triphosphates (XTP). There is an obligatory first step with ATP β − γ bond hydrolysis, followed by phosphodiester bond formation. The first recognizable initiation complex is formed after formation of a trinucleotide (A-X-X). Elongation factors, such as TFIIS, associate with the elongating complex (of unknown composition), RNA is elongated, and eventually there is termination of transcription. A complex of unknown composition remains bound to the DNA and participates in preferential reinitiation of transcription on the same template.[376] Control may be exerted at several distinct steps (see Section IV.B).

Several observations suggest that regulation may be exerted directly on general transcription factors. The AdE1a gene products produced early in viral infection can trans-activate transcription from a variety of promoters and in several cases the TA motif is the target for trans-activation.[186] Similarly, the TA motif is one of the targets for trans-activation of the herpes simplex virus (HSV) thymidine kinase gene by viral immediate early gene products.[187] The TA motif and startsite region is sufficient for fully regulated HSV late gene transcription.[188] A small fragment of the chicken conalbumin promoter, extending from −45 to +62, is sufficient for tissue-specific transcription.[189]

3. Regulation

At what steps in the basic reaction might regulation be exerted? Several observations suggest that the slow formation of the preinitiation complex may be such a step. In the ADMLP promoter, a specific TF binds to sequences upstream from the TATA box, and the bound factor increases the rate of preinitiation complex formation.[156] Similarly, the specific TFs which bind to the upstream region of the silk fibroin gene stimulate preinitiation complex formation.[190] However, subsequent steps in the initiation reaction may also be controlled. This is suggested by in vivo experiments on the *Drosophila* hsp70 gene, which in the uninduced nontranscribed state has proteins bound to the −40 to +60 region of the promoters.[152] Pol.B is associated with this complex.[153] Heat shock rapidly stimulates transcription from the promoter, through association of a TF with the upstream region.[152,155] The simplest interpretation of these results is that an inactive stable preinitiation complex is present on the promoter, and heat-shock TF binding activates the complex. Similar considerations may apply to the clustering of pol.B at the 5′ end of several other genes.[191-193]

The mechanism of basal transcription mainly has been studied in vitro, and it should be emphasized that in general the startsite selection region requires additional elements for efficient transcription in vivo. These additional elements are the major determinants of the efficiency of transcription and they probably serve to activate a zone of DNA with which general transcription factors can interact. The general transcription factors are probably responsible for subsequent fine-tuning which determines both the precise site(s) of initiation of transcription and final efficiency of transcription (see Section IV.D).

C. Upstream Regions and Enhancers

Upstream regions and enhancers are distinguished functionally by the inability of upstream elements to stimulate transcription when moved away from the selector region, while enhancers are capable of stimulating transcription at a distance. This raises the possibility that there are different upstream element and enhancer TFs which activate transcription by different mechanisms. Recent evidence suggests rather that many, if not all, TFs are capable of both short- and long-range activation and that such a distinction may not exist.

Many TFs which interact with TEs in upstream regions can stimulate transcription from distantly located promoters. In the SV40 early gene promoter, there are Sp1 binding sites (GC motifs) in the upstream element. This upstream element is much less efficient in stimulating transcription when moved away from the selector region (e.g., see Reference 194). However, the SV40 upstream element is also part of the divergently transcribed late promoter, and Sp1 stimulates transcription from the later startsites, which are about 200 bp away (see Section IV.D). Sp1 binding sites are located up to 250 bp upstream from the startsite in various promoters,[195] and are also found in enhancers, e.g., the mouse metallothionein I enhancer.[196] The ADMLP upstream TF stimulates transcription from the divergent ADMLP and IVa2 promoters. Its binding site is located in the upstream element, around −60 bp from the major late startsite and at −160 from the IVa2 startsite.[197] The Ig dc-octamer motif is present in both upstream elements (IgH and Igκ promoters) and enhancers (SV40, IgH, and *Xenopus* U2 gene; for a review, see Reference 198). A single dc-octamer motif does not efficiently stimulate transcription at a distance, thus behaving as an upstream TE. However, a functional enhancer can be generated by multimerizing this motif.[199] The heat-shock TE from the *Drosophila* HSP70 gene, which was originally identified as an upstream element because it is inactive when distantly located, can also act as an enhancer when multimerized.[200] Functional heat-shock TEs are sometimes located at some distances from the startsite (up to 250 bp[201]). Generation of enhancer activity by duplication is a widespread phenomenon,[202-204] suggesting that a large number of TEs have the inherent property to activate transcription at a distance. It remains to be seen if there are any TFs which can only stimulate transcription initiation over short or long distances, and which may hence have only upstream or enhancer-like properties.

The SV40 and other enhancers share the property of upstream elements of preferentially activating a proximal promoters.[205,206] There does not appear to be an upstream element in the SV40 enhancer because the same DNA sequences are required for both short- and long-range activation of transcription.[26] The SV40 enhancer can functionally replace a heterologous upstream element[207] or act as an upstream element for substitute TATA-like sequences.[208] Enhancers and upstream elements share many other properties, such as bidirectional stimulation of transcription, ability to modify chromatin structure, and a role in regulation of both the level and tissue specificity of transcription (e.g., see Section IV.D).

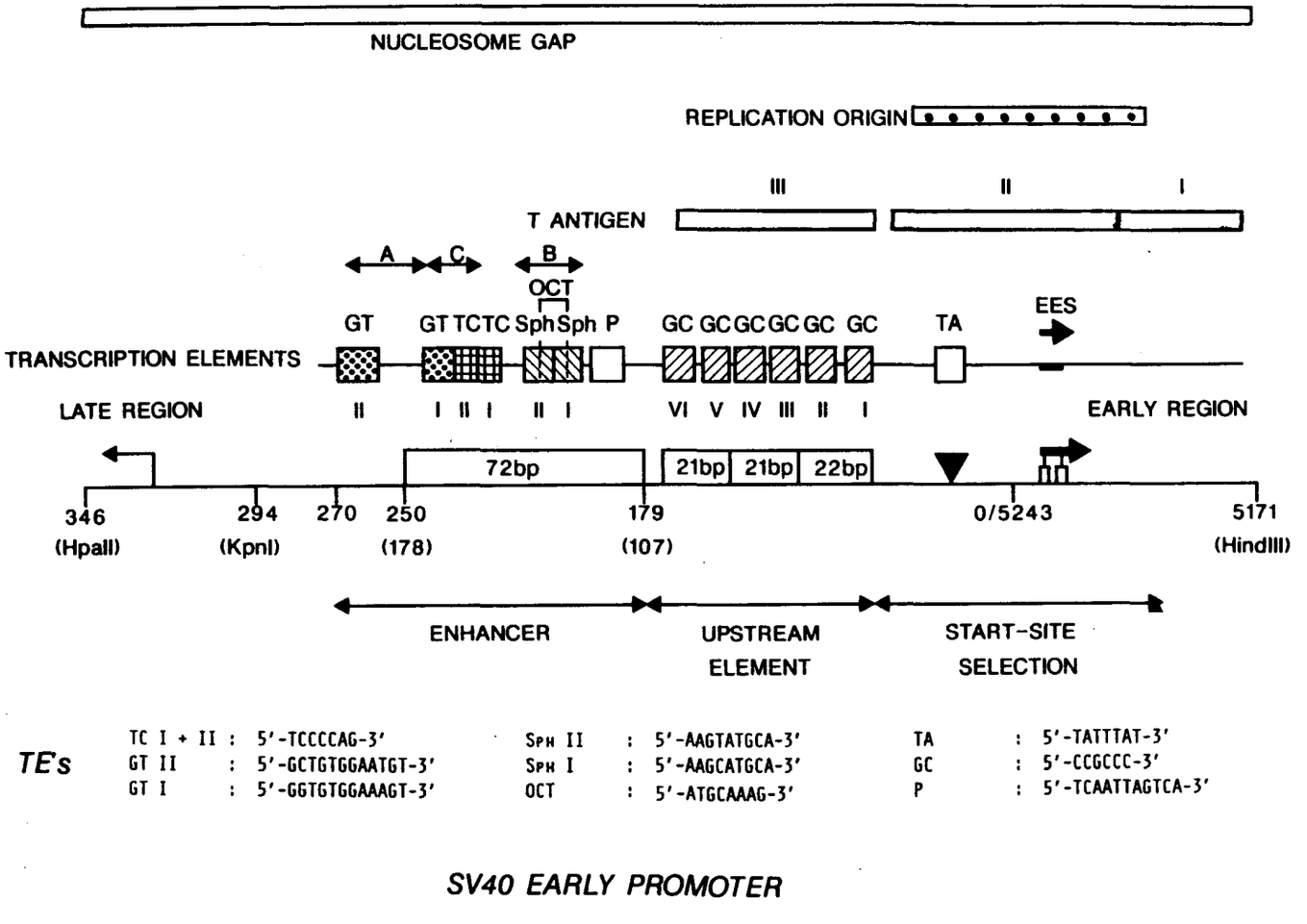
A reasonable interpretation of these results is that many aspects of the mechanism of transcription activation by proximal and distal elements is similar, and the difference is the advantages that proximity itself can confer. For example, for protein-protein interactions, if the proteins are correctly aligned in close proximity on the DNA, then interactions between them would be favored compared to when looping would be required to bring them together. Similar considerations may apply for transcription activation through DNA structural modifications in that it may be easier to modify the structure of the DNA sequences close to a TF interaction site compared to distantly located sequences which would require propagation of the signal along the DNA. Proximal TEs can be considered therefore to be in a privileged position, so that moving them away will often, if not always, decrease their effect. This would help to explain why promoters have proximal elements even if they do not have distal elements, especially in cases where there is a premium for space and/or rapid evolution, such as in bacteria or in viruses.

D. An Example of Promoter Organization: The SV40 Early Promoter

1. The Selector

Transcription of the SV40 early genes early in viral infection leads to production of T antigen, which represses the early promoter and regulates many other features of the viral life cycle.[209] The early promoter has been studied extensively, mainly using transfection assays with recombinants containing the early promoter linked to heterologous structural gene sequences and in vitro with cell extracts. The early promoter is about 300 bp long and consists of 3 regions: the selector region, the upstream element containing the 21-bp repeats, and the enhancer containing the 72-bp repeats (see Figure 7: only 1 of the 72-bp-repeated sequences is shown). The selector and upstream elements overlap with sequences required for T-antigen binding and DNA replication.

There are several major and some minor startsites for transcription from the early promoter early in infection [early-early startsites (EES), see Figure 7], and about 30 bp upstream from the major EES there is a TA motif 5′-TATTTAT-3′ embedded in an A-T stretch of DNA. Deletions in the selector region and point mutations in the TA motif have only a small effect on transcription efficiency (about fivefold),[210-213] showing that this region contributes little to overall promoter efficiency. A double point mutation in the TA motif decreases transcription from the major EES and increases transcription from minor sites.[213] Insertion of heterologous sequences (10 to 120 bp) between the upstream and startsite selection region results in a decrease in initiation from the EES and the appearance of new startsites 30 to 60 bp downstream from the 21-bp repeat.[214] Either inserting or deleting 5 bp of DNA between these promoter elements results in an increase in transcription from minor startsites and a decrease in initiation from the major startsites, whereas inserting 10 bp regenerates the original pattern of startsites.[215] These results suggest that the upstream elements activate a region close by with which general transcription factors can interact, and that within this region there are a number of different sites of interaction which are in competition with each other for a limiting number of transcription factors. Two determinants contribute to the selection of the actual sites of initiation: the possible sequences of the activated region with which general transcription factors can interact, and the stereospecific alignment of these



SV40 EARLY PROMOTER

FIGURE 7. The SV40 early promoter. The regions of the early promoter, including the startsite selection region, the upstream element (21-bp sequences), and the enhancer (72-bp sequence) are shown. The location of transcription elements, the region of the enhancer which when duplicated generates an active sequence (A, B, C),[377] and the major early-early startsites (EES) are shown. The coordinates of key features, such as restriction sites and the limits of the enhancer, are shown. The coordinates in parentheses are the boundaries of the deleted 72-bp sequence. Also illustrated are the location of T-antigen binding sites I, II, and III, the origin of DNA replication, and the location of the nucleosome gap.

sequences with upstream sequences. There is a preferred interaction with the TA motif indicated in Figure 7, and changing either its sequence or its alignment with the 21-bp repeats favors minor interactions and startsites. Whereas the consensus TA motif has G-C-rich surrounding sequences (see Section IV.B), the SV40 TA motif is part of the an A-T-rich sequence. Presumably, these A-T-rich sequences act as alternative TA motif recognition sequences for general transcription factors and result in initiation from minor startsites. The A-T-rich sequence is important for DNA replication.[216] Strikingly, the point mutation in the TA motif which decreases transcription from EES also inhibits replication,[213] showing that the TA motif has a dual function, and suggesting that the sequence organization of this region is a compromise between requirements for replication and transcription.

2. The 21-bp Repeats, GC Motifs, and Sp1

The 21-bp repeats contribute to the efficiency of the SV40 early promoter. Complete deletion decreases transcription efficiency about 100-fold, whereas partial deletions have intermediate effects, suggesting that the repeated sequences complement each other.[217-219] The GC motif, 5′-CCGCCC-3′, in the 21-bp repeats, is required for both promoter activity and binding of the transcription factor Sp1. It is present 6 times, twice in each 21-bp sequence (see Figure 7). Systematic mutagenesis has shown that there is a gradient of importance of the GC motifs, with the motifs closest to the startsite selection region contributing most to early promoter activity.[214,220] The order of binding affinities for Sp1, III > V > II > VI > I > IV, results from both differences in the sequences flanking the GC motifs, which can alter the affinity by over an order of magnitude, and from steric constraints, mainly between sites IV and V.[121,214,220] The weak SP1 binding site I is most important for early transcription, suggesting that its position relative to the startsite, and the possibility to interact with general transcription factors, is important for Sp1 activation of transcription. The multiplicity of binding sites may serve to increase upstream element efficiency.

Sp1 is an abundant protein; there are 5,000 to 10,000 molecules in a HeLa cell, and it is found in a large number of different cell types (for reviews, see References 195, 221, and 222). It serves as a TF for a number of cellular genes, although an insufficient number of promoters are known to try to establish a physiological relatedness between them. Sp1 binding sites are found interspersed with other TEs, suggesting that it can interact with a variety of other TFs besides those found in the SV40 early promoter. Sp1 binding sites are found in single or multiple copies, in either orientation, and at various distances up to −250 bp upstream from the startsite. In SV40, Sp1 stimulates both early and late transcription, whose startsites lie at different distances and in opposite orientations. In addition, it still functions when its binding site is inverted even though there is apparently no symmetry in the GC motif.[219,223,224] This suggests that there is considerable flexibility in the way Sp1 interacts with other TFs. However, stereospecific alignment with the startsite selection and enhancer regions is required for maximal transcription efficiency,[215] suggesting that Sp1 interacts with other TFs by protein-protein interactions.

How does Sp1 stimulate transcription bidirectionally, even though the GC motif appears not to be symmetrical? There are various ways in which this could occur: (1) Sp1 may have two symmetrically disposed interaction sites and (2) a bidirectional interaction site could be created by having two functional domains linked by a flexible stem, one which binds DNA, and another which interacts with other TFs. Such separable domains for DNA binding and transcriptional activation exist in repressor proteins of *E. coli* λ phage[135] in several yeast TFs[225,226] and perhaps in several hormone receptors in higher eukaryotes (for a review, see Reference 227). In agreement with this possibility, there is an Sp1 proteolytic subfragment which displays the same affinity and specificity as the intact protein, but is less potent in transcription activation.[121]

How may Sp1 achieve broad specificity in its interaction with other TFs? Transcription-

activating segments of two yeast TFs are acidic and have no obvious sequence homology.[226,228] Several bacterial repressors which are believed to contact RNA polymerase have acidic amino acids required for transcription activation.[229] Perhaps the build up of a large acidic region can provide a nonspecific way in which many different transcription factors can collaborate together to build up an active promoter complex.[229]

3. The SV40 Enhancer

a. The Minimum Enhancer Sequence

The SV40 72-bp repeat contributes most to the efficiency of the SV40 early promoter. Its deletion results in a 400-fold decrease in transcription.[26] One copy of the 72-bp-repeated sequences can be deleted without substantially affecting either viability of the virus[230-232] or enhancer activity. The minimum enhancer consists of about 100 bp, extending between nucleotides 179 and 270 (see Figure 7).[26,233,234] However, there are sequences beyond nucleotide 270, between positions 298 and 347, which can double enhancer activity.[26,203,235]

The term "enhancer" originally arose from the observation that the 72-bp repeats could stimulate transcription even when located at considerable distances upstream or downstream from either the rest of the SV40 early promoter or heterologous promoters.[194,236] At that time, this was a remarkable property compared to other known promoter elements. However, it is now known that many TFs, if not all, show considerable flexibility in the distance over which they stimulate transcription, particularly when present in multiple copies (see Section IV.C). In addition, it should be emphasized that SV40 enhancer activity is diminished at distant locations,[26,205,208] and that even a 5-bp displacement of the 72-bp sequence decreases promoter efficiency 6- to 9-fold.[215]

b. Enhancer TFs and Synergism

The minimal SV40 enhancer contains at least five different TEs: two in single copies (the P and dc-octamer motifs) and three repeated copies (Sph, TC, and GT motifs).[26,124,204,237] Each TE interacts with a TF,[104,124,238,239] and all the TFs appear to contribute to enhancer activity since point mutations in each motif diminish promoter activity and prevent TF binding.[26,104] Each TE contributes only partially to overall enhancer activity, since mutation of a motif decreases promoter activity at most 8-fold, and the mutated enhancer still stimulates transcription 50-fold.[26] In addition, each motif does not contribute equally to promoter activity. For example, in HeLa cells, mutations in TCI have a less striking effect than do mutations in TCII.[26] The enhancer cannot be considered to be built up by the simple addition of several individual elements, but rather there is synergistic cooperation between a minimum number of components to constitute an active element.

Either left- and right-hand halves of the enhancer (A 179 to 225, B 229 to 280)[26] or smaller parts[204,237] are almost totally inactive alone. Enhancer activity can be restored by duplication or further multimerization of the individual parts.[203,235,240,241] However, once an active minimal element is restored, further multimerization has an additive effect[26] up to a certain point.[242] In studies of revertants of enhancer mutant virus, it was shown that duplication of either of three elements (A, B, or C in Figure 7) corresponding to motifs GTII, GTI + TCII, or SphI + II + OCT, respectively, regenerates viable virus.[237] Oligonucleotides corresponding to these regions can restore active enhancers when multimerized.[204] These results suggest that either multimerization of an individual TE or association between different TEs is required to generate an active enhancer. Two copies of a TE may be insufficient in many cases, since the two inactive halves of the enhancer (A and B in Reference 26) each contain a duplicated TE (Sph in A, both GT and TC in B). Duplicating A or B or putting A and B together generates a very active element, suggesting that multiple copies of some motifs are required for synergism, but that the type of motif is not particularly important.[26] The underlying mechanism for the burst of promoter activity is unknown at

present. Synergism is seen whether the enhancer is close by or far away,[26] suggesting that the cooperative effect is not related to differences in short- or long-range types of enhancer activation.[205] However, synergistic interactions distinguish the SV40 enhancer from the rest of the promoter, since synergism is observed between the two halves of the enhancer, but not between either half and the 21-bp repeats.[27]

c. Cell Specificity

The SV40 enhancer is active in a wide variety of cell types, but does show some cell-type preference. This broad specificity results from the combinatorial specificities of the individual elements, which, rather than all having similar specificities as the whole enhancer, have complementing specificities. This has been shown using both the whole enhancer, and multimers encompassing individual TEs. Multimers of the GTI + TCII motif are active in most cell types, multimers of GTII are active only in CV1, and multimers of SphI + II are lymphoid-cell specific.[204] Using the complete enhancer, it has been shown that different factors in different cell types interact with overlapping motifs to activate transcription. The Sph motifs interact with factors in HeLa cells, and are required for transcription activation.[124] In myeloma cells, a different motif, 5′-ATGCAAAG-3′, created by the overlap of the juxtaposed Sph motifs (see Figure 7), interacts with a different TF. In addition, the proteins binding to GTII are different in HeLa cells and BJA-B lymphoid cells.[124] These results suggest that the broad specificity of the SV40 enhancer results from the presence of both TEs which are active in a wide variety of cell types, and TEs which permit use of different cell-specific TF in different cells. It can be predicted that duplication of sequences, such as that of the 72-bp sequences in some viral strains, should also help create a broad cell specificity, since even if some TEs are inactive in certain cell types, any repeated TEs which are active could restore enhancer activity. This principle is illustrated by the polyoma-virus enhancer, which contains multiple nonduplicated enhancer elements[241,243] with different cell specificities.[243] Duplications of certain regions of the enhancer are responsible for altered cell specificities.[244]

d. SV40 Enhancer TFs Are Found in Other Promoters

The TEs in the SV40 enhancer are also found in other enhancers. The P motif is highly homologous to a sequence in a 26-bp fragment of the polyoma-virus enhancer which is essential for enhancer activity,[241] and this motif is duplicated in a number of variant viruses which are selected for growth in PCC4 teratocarcinoma cells.[245] Sequences similar to the Sph motif are found in BK virus, lymphotropic papovavirus, and the κ light chain enhancer (for bibliography, see Reference 26). The dc-octamer motif is present in many promoters, especially Ig promoters and the IgH enhancer (see Section IV.E). The TC motif is found in the Igκ light-chain enhancer[128] and the H-2K^b enhancer.[238] In vitro studies have shown that the binding site for this factor in the H-2K^b promoter, 5′-TGGGGATTCCCCA-3′, is an inverted repeat of the TC motif 5′-TCCCCA-3′. The SV40 sequence 5′-TGGAA-AGTCCCCA-3′ is not symmetrical, which may explain why the binding affinity for the H-2K^b sequence is five times greater. Several other TFs bind to palindromic sequences (CTF/NF1;[246] ADMLP;[122,247] c-fos serum inducible factor[248-250]), and with lower affinity to a single motif.[246] Many prokaryotic regulatory proteins recognize palindromic DNA as dimers,[251] suggesting that some eukaryotic TFs may also bind as dimers. In addition, the TC factor may be involved in α-interferon regulation of H-2K^b transcription[238] and B-cell-specific enhancer activity (see Reference 128 and Section IV.F). The GT motif overlaps the core motif of Weiher et al.[252] and is found in a large number of viral and cellular enhancers (for a compilation, see Reference 253). Despite the sequence similarities between the TEs GTI and GTII, the factor which interacts with GTII in HeLa cells is absent in lymphoid cells, while the GTI factor is present in both cell types,[124] and multimers of either motif have

different specificities.[204] This suggests that the factors interacting with GTI and GTII may be different, although related. Similar families of related TFs are known, such as the hormone receptors.[227]

SV40 enhancer TFs stimulate transcription from both the early and late promoters,[254-256] suggesting that there is flexibility in the types of interaction which exist between the enhancer and other SV40 TFs. In addition, the SV40 enhancer TFs are associated with a number of different TFs in other promoters. It remains to be seen what restrictions apply for interactions between TFs. That there are restrictions is suggested by the observations that the IgH enhancer stimulates a homologous promoter more efficiently than a heterologous promoter,[257] and that the SV40 enhancer stimulates certain transcription units with different efficiencies.[258]

e. The Multifactor-Promoter Complex

How do the SV40 TFs interact to generate an active transcription complex? Since stereospecific alignment is required between the enhancer and the 21-bp repeats, between the 21-bp repeats and the selector, and between individual parts of the enhancer (A and B[26,380]), for maximum promoter activity, and since the enhancer and Sp1 TFs interact with mainly the same face of the DNA helix,[104,215,222] it has been suggested that the enhancer TFs form part of a broad complex of interacting proteins located mainly on one side of the double helix. Such a complex could efficiently compete with the rest of the genome for limiting general TFs. Since the same TFs are required for short- and long-range activation, a similar complex may form when the enhancer is distantly located,[215] by looping out of intervening DNA (see Section III).[135] There is a large increase in enhancer activity as the spacing between the enhancer and the rest of the promoter is decreased below about 150 bp.[205] This effect can be explained by the ability of closely apposed TFs to interact directly, thereby not requiring bending of a short stretch of rigid DNA helix when the promoter elements are separated by about 150 bp of DNA (see Section III.B). However, the looping model also suggests that the structure of larger lengths of intervening DNA between an enhancer and the rest of the promoter should not influence enhancer activation. Psoralen monoadducts (without interstrand cross-links) in the intervening DNA inhibit SV40 enhancer activation of a distant β-globin promoter, which would argue in favor of a model in which TFs slide along the DNA between the enhancer and the promoter.[259]

f. Enhancers as Transcription Switches

It has been suggested that enhancers are required to switch on transcription, and that once transcription is activated, genes remain actively transcribed whether or not the enhancer is present. This would explain certain results. Competition for SV40 enhancer TFs decreases transcription before but not after the establishment of stable transcription complexes,[260] suggesting that the SV40 enhancer is not continuously required for transcription. The IgH enhancer is required to activate transcription in transfection experiments, while in several B-lymphoid cell lines in which the IgH enhancer is deleted, IgH gene transcription remains normal.[261,262] Presumably, the enhancer switched on transcription before it was deleted. Igκ light-chain enhancer activation accompanies the pre-B-B cell switch and activation of κ light-chain gene transcription.[263,264] Some mature B-cell variants maintain efficient κ gene transcription despite the loss of active Igκ enhancer TFs which inactivate the Igκ enhancer (as measured by transfection experiments). This again suggests that enhancer function is not required for maintenance of active transcription.[265] However, models which explain these results have to take into account that enhancer TFs remain bound to the DNA[94] even though the enhancer is apparently dispensable, and that the activity of some enhancers is rapidly and fully reversible (e.g., the glucocorticoid-dependent enhancer).[266,267]

4. Chromatin Structure

Late in SV40 infection the SV40 genome is organized into a typical nucleosomal structure, and about 20% of these minichromosomes have a nucleosomal gap extending through the early and late promoters, which is sensitive to nuclease digestion (see Figure 7).[268-274] Transcriptionally active minichromosomes, which represent about 1% of SV40 molecules, contain the gap.[275,276] How is the gap formed, and what is the function of the gap?

Studies on in vitro assembly of nucleosomes on SV40 DNA have shown that at low histone/DNA ratios, nucleosomes form poorly on the origin region compared to other sequences, while at higher ratios, nucleosomes will form on this region.[277,278] Studies with SV40 viruses with two origins — one to provide vital functions, the other for manipulation — have shown that either the 21-bp repeat or the enhancer, but not the origin of replication-transcription startsite selection region, is sufficient to generate both nuclease-sensitive regions on the DNA and a gap.[274,279,280] The proteins associated with the gap have been the subject of several studies. In isolated nuclei, about 20% of the SV40 molecules contain a protein bound to the GC motifs. From its abundance, it was suggested that the protein may be involved in gap formation. It seems unlikely that the protein is Sp1 because its protection pattern is different. In another report, T antigen was the only protein detected on the nucleosome-free origin of minichromosomes. It was located mainly over the 21-bp repeats.[282] It remains to be seen whether T antigen, TFs, or other factors contribute to formation of open chromatin. It has been suggested that the nucleosome gap may be an "open window" which allows access of transcription factors to the DNA[274] and, indeed, the chromatin structure of SV40 minichromosomes isolated late in infection favors in vitro transcription from the late promoter over the early promoter, whereas on naked DNA the early promoter early promoter is preferred.[283] The chromatin structure of the transcription complex early in infection remains to be established. It should be emphasized that the early promoter has been studied mainly using transfections, and a typical chromatin structure may not form on transfected DNA (see Section II.B).

5. Matrix Attachment

Cellular DNA is organized into a matrix structure, and a large body of indirect evidence suggests that association with this nuclear matrix can affect both transcription and replication (for a review, see Reference 4). For example, matrix attachment regions in the IgH and Igκ gene loci have been mapped in close vicinity to the enhancers,[284,285] suggesting a close association between transcription control and nuclear organization. SV40 associates with the nuclear matrix both early[286] as well as late[287] in infection, implicating matrix attachment with at least some viral functions.

6. Torsional Stress

Torsional stress introduced by DNA gyrase, a type II topoisomerase, affects the activity of several prokaryotic promoters.[288,289] Studies using novobiocin, an inhibitor of *E. coli* DNA gyrase and eukaryotic DNA topoisomerase II, have led to the proposal that topological tension is important for transcription in eukaryotic cells (for bibliography, see References 290). However, in eukaryotic cells, there appears to be little topological tension in the DNA,[291] and up to now no detectable gyrase activity. In addition, the use of novobiocin as a specific inhibitor of DNA topoisomerase II has been questioned.[292-294] These uncertainties extend to SV40. Supercoiled DNAs, and especially those that carry the SV40 enhancer, yield in transfections higher levels of expression than linearized DNAs, suggesting that efficient expression depends upon DNA topology.[295] A major topoisomerase II cleavage site is located close to the SV40 enhancer (around nucleotide 270).[296] It has been reported that 2 to 5% of SV40 minichromosomes, including the transcriptionally active molecules, are torsionally strained.[297,298] However, in another study, no topological strain was detected in

mRNA producing viral transcription complexes.[299] In transfection studies, the SV40 enhancer efficiently stimulated the β-globin promoter despite that fact that it was topologically uncoupled while physically linked, arguing against a role of topological strain in enhancer function.[300] The role of topological stress in early promoter activity remains uncertain at the present time.

7. DNA Structure

The structure of SV40 early promoter DNA before or after formation of the transcription complex is unknown. Various studies have suggested that SV40 early promoter DNA can adopt either a Z or a bent structure, which may affect promoter activity. Negatively supercoiled SV40 DNA binds anti-Z-DNA antibodies preferentially between map positions 40 and 474, which overlaps the 21- and 72-bp repeat elements of the early promoter.[301] In addition, Z-DNA binding proteins isolated from SV40 minichromosomes bind the Sph region of negatively supercoiled DNA.[302] Alternating purine-pyrimidine sequences with the potential to adopt a Z-DNA structure overlap the SphI and GTII motifs.[26] However, mutation in these motifs, which retain an alternating sequence, inhibits transcription, showing that the potential to form a Z-DNA structure is insufficient for transcription activity.[26] Bent DNA can result from either the sequence of the DNA[303] or binding of TFs.[304] SV40 early promoter activity is inhibited by T-antigen binding to sites I and II (see Figure 7).[305-309] An altered DNA conformation, presumably a bend, is required for T-antigen binding to region I.[310] The sequence 5′-AATTTTTTTT-3′, immediately upstream from the TA motif 5′-TATTTAT-3′, adopts a bent DNA conformation,[311] suggesting that the SV40 DNA which is recognized by general TFs may not naturally have a classical B-type helical structure.

8. Transcription and Replication

Transcription from the SV40 early region provides T antigen required for replication, repression of early transcription, and stimulation of late transcription.[312,313] The 21- and 72-bp repeats are required for efficient replication[314-318] as well as late transcription.[219,223,224,254-256] An overlap between sequences required for replication and transcription is also found in both the polyoma-virus enhancer,[241] and adenovirus 2 (AD2).[246,319] AD replication requires two factors: NF1, which is the same factor as the TF CTF which interacts with a number of promoters (such as herpes thymidine kinase, α globin, β globin, and H-ras),[246] and NFIII, which is similar to the dc-octamer TF (see Section IV.E). It remains to be established whether any of the SV40 early promoter TFs are also required for replication. A goal for the future is to establish how the same factors lead to regulation of transcription, replication, and other functions involved in the viral cycle.

E. Tissue-Specificity: The Immunoglobulin Heavy (IgH)- and κ Light (IGκ)-Chain Promoters

B-lymphoid-specific expression of the IgH and Igκ genes is regulated in part at the transcriptional level by both positive and negative mechanisms. The IgH and Igκ promoter elements have been studied extensively, and provide a good example of the mechanism of tissue-specific transcriptional control (for a review, see Reference 320).

The Ig genes are assembled from multiple germline segments during development.[321] For the IgH genes, one of the large number of variable (V) gene segments is joined to one of the diversity (D) and joining (J) segments, and to the Cμ constant region (see Figure 8A). Later in development, class switching takes place by rearrangements between the switch (S) regions which replace Cμ by other constant regions. The intron between J and S, which normally is not deleted during these rearrangements, contains the IgH enhancer (for bibliography, see Reference 8; also see Figure 8), which is specifically active in B-lymphoid cells.[8,79,82] Upstream from each V gene segment lies a tissue-specific promoter element

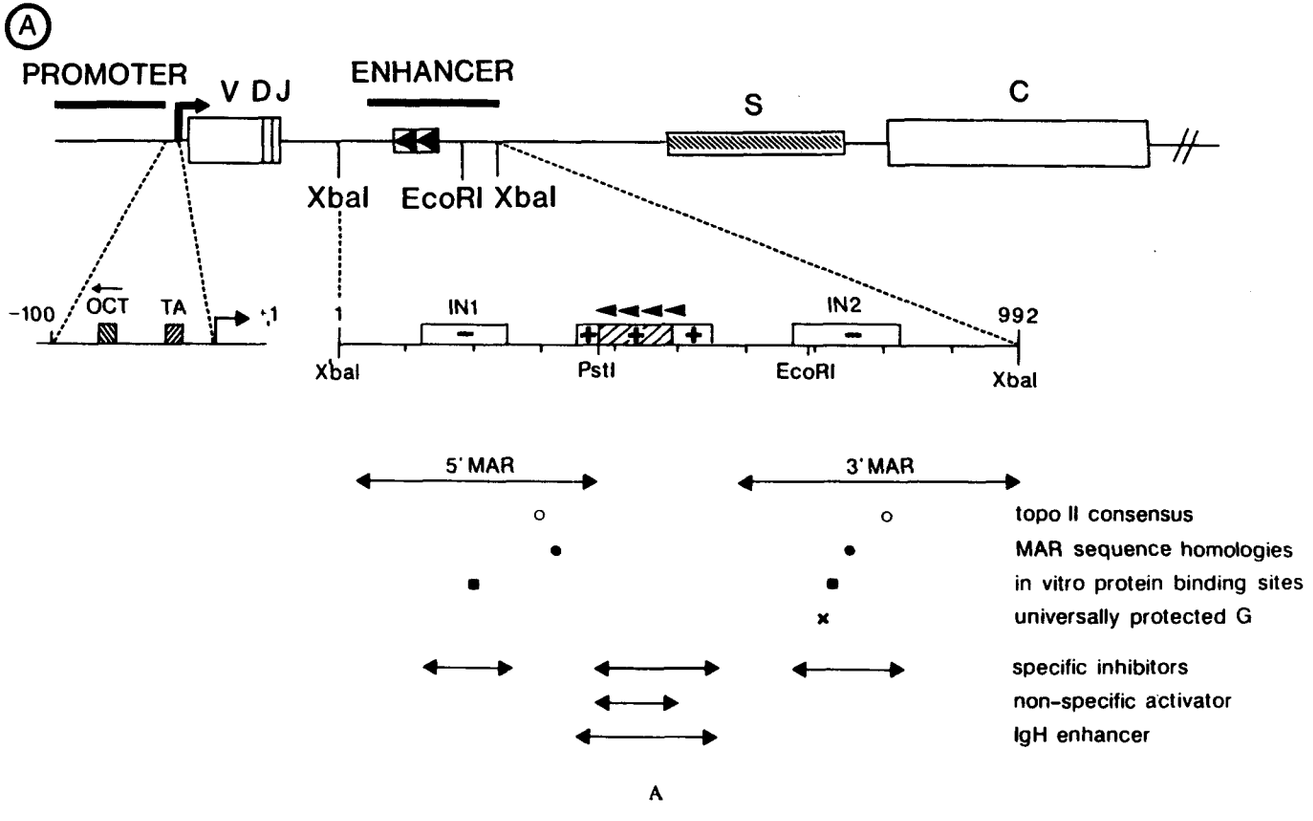


FIGURE 8. The immunoglobulin heavy-chain (IgH) gene promoter elements. A rearranged IgH gene is shown, including the variable (V), diversity (D), joining (J), switch (S), and constant (C) region segments. The upstream promoter includes a TATA-like sequence (TA) and a dc-octamer motif (OCT — the arrow indicates the orientation of the motif). The IgH enhancer is represented schematically (A), and the sequence is shown (B). Also shown are the locations of matrix attachment regions (5′ and 3′ MARs), topoisomerase II consensus sequences (topo II consensus), MAR homology sequences,[285] binding sites for proteins in in vitro extracts,[325] a G base protected against DMS modification in many cell types (universally protected G),[94] and both specific inhibitory and nonspecific activatory sequences.[206,323,242] On the sequence of the IgH enhancer (B) are indicated the Ephrussi motifs (E1 to 4),[94] three sequence homologies to core sequence (CORES 1 to 3),[252] and the dc-octamer (OCT — the arrow indicates the orientation of the motif). Also indicated are protein binding sites identified in vitro, including NF-μE1, NF-μE3,[324] LN1-5 and LC1-3,[108] Ig PE-1-3,[127] B, C1-3,8,[325] and cell-specific and nonspecific enhancer sequences.[206,323]

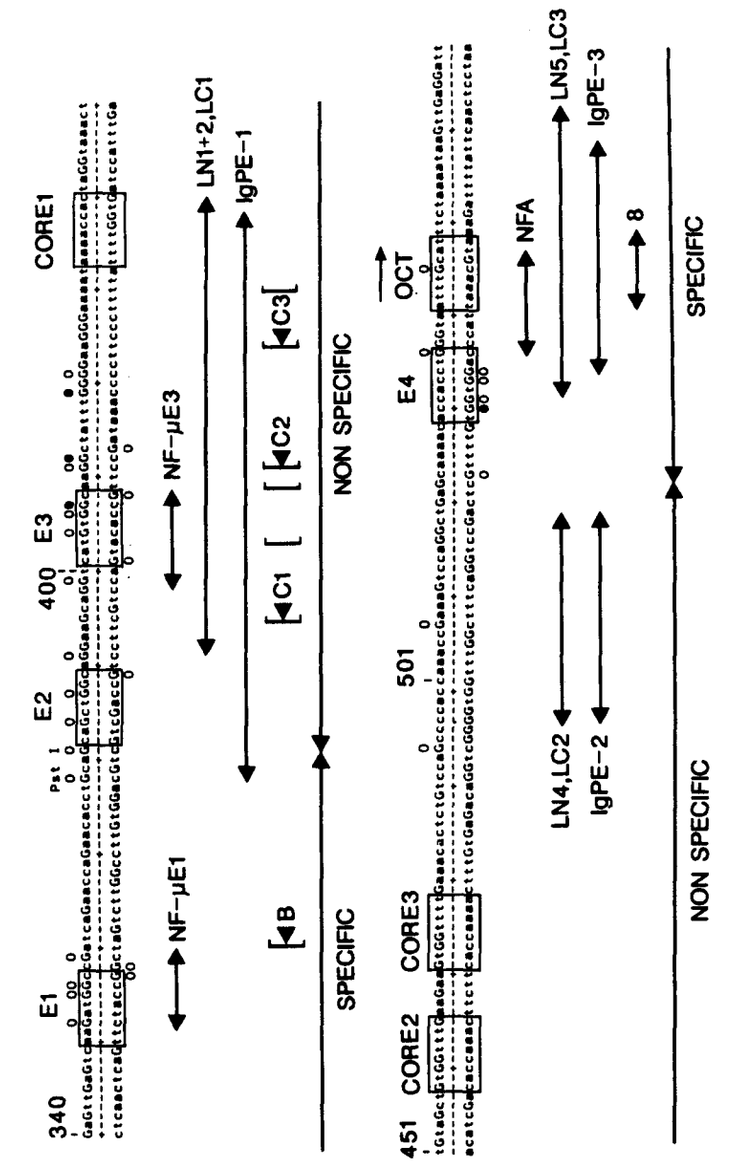


FIGURE 8B.

which extends about 100 bp upstream from the startsite, and includes both a TA motif and an upstream region.[62,322]

The proteins that interact with the IgH enhancer were first identified by genomic footprinting (see Section II.B).[94,96] From the pattern of modified sensitivity of G residues to DMS methylation, it was predicted that there is a single B-lymphocyte-specific factor which interacts with four similar E motifs (E1 to 4, Figure 8). More recent studies show that the mechanism by which tissue specificity is achieved is more complex. In particular, there are at least five different factors that interact with the enhancer, and some of them are present in many cell types. In addition, negative as well as positive regulation contributes to the tissue specificity.

In transfection experiments it was shown that subfragments of the IgH enhancer have different cell specificities, and in particular a central element is active in nonlymphoid cells, whereas flanking sequences are tissue specific (see Figure 8B).[206,323] These results suggest that there are TFs in non-B cells which can interact with the IgH enhancer, and that flanking sequences contribute in either a positive or negative fashion to tissue specificity. Point mutations in E2, E3, and in one motif that is homologous to the Weiher GT core sequence (core 2^{381}) as well as a deletion from 490 to 520 (see Figure 8 and Reference 206) decrease transcriptional activation in lymphoid and nonlymphoid cells, suggesting that the corresponding TFs are not B-cell specific. Sequence-specific DNA binding proteins have been identified in in vitro extracts which bind to E2,[127] E3,[108,127,324,325] core 1,[108,127] and the 495 to 520 region.[108,127] Only small differences were detected in the protection patterns in lymphoid and nonlymphoid cells,[108,325] supporting the suggestion that the TFs which interact with E2, E3, core 1, and the Ig PE2 or LN4-LC2 regions are present in many cell types. Factors binding to Ig PE1 and Ig PE2 also bind to the polyoma and MSV enhancers, and the lymphotrophic papovavirus (LPV) enhancers, respectively, which are not B-lymphoid cell specific.[127] The E3 binding TF also appears to bind to the E3-like motif in the Igκ enhancer (see Figure 9).[128]

Deletion of sequences both upstream and downstream from the central region decreases enhancer activity specifically in B cells.[206,323] The upstream region contains the E1 motif (see Figure 8). Cell extracts contain at least one protein which interacts with E1 and which is different from the factor that interacts with E3 (NF-μE1 and NF-μE3 in Figure 8B).[324,325] The role of this protein in positive and/or negative regulation remains to be established. The downstream region contains the E4 and dc-octamer motifs. Transfection studies with point-mutated DNA fragments have shown that the dc-octomer motif, and to a much lesser extent the E4 motif, activate transcription exclusively in B cells,[199,382] suggesting that the dc-octomer motif is a major element determining in a positive manner the tissue-specific activity of the IgH enhancer. The same motif is present upstream from the TA motif in both the heavy- and light-chain promoters (for a recent review, see Reference 198) and is sufficient for their tissue-specific activity.[62,322,326-330]

Cell extracts contain both lymphoid-specific and nonspecific factors which interact with the dc-octamer motif.[108,127,130,199,325,331-336] It seems likely that only the lymphoid-specific factor can activate the IgH and Igκ promoter elements, and that the nonspecific factor activates other promoters which have dc-octamer motifs that are not B-cell specific, such as H2B,[335] the SV40 enhancer,[337] various U snRNAs,[338] and HSV thymidine kinase.[339] A plausible model for the cell specificity is that the nonspecific dc-octamer factor can only interact with TFs present in the nonspecific promoters. The availability of in vitro extracts which reproduce cell-specific Igκ promoter activity should help answer this question.[109] dc-Octamer-like motifs are found in a variety of eukaryotic genes and some prokaryotic promoters,[198] and a factor binding to this motif is present in yeast.[332] These results suggest that there is an evolutionary and functional relationship between transcription systems in widely different organisms. In addition, a similar factor may stimulate replication of adenovirus

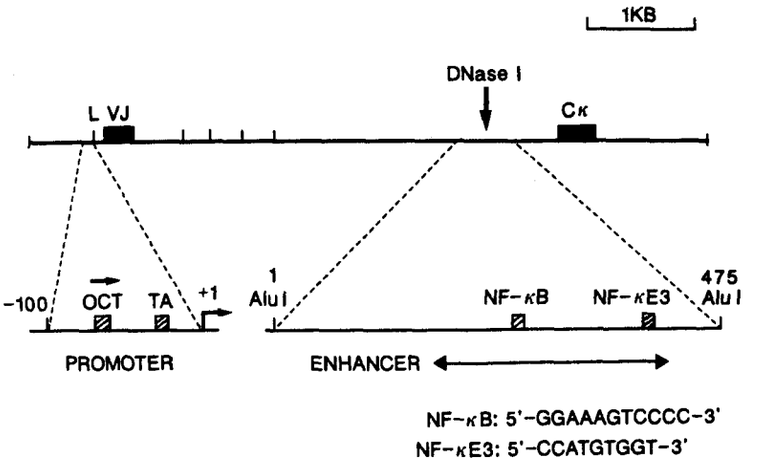


FIGURE 9. The immunoglobulin κ light-chain (Igκ) gene promoter elements. A rearranged Igκ gene is shown, including the leader sequence (L), the variable region exon (V), the joining segment (J), and the Cκ constant region. The DNAse I hypersensitive site is located in the Igκ enhancer which contains two identified TEs, for NF-κB and NF-κF3 (see Section IV.F). The promoter element upstream from the startsite contains TA and dc-octamer (OCT) motifs. The arrow above OCT indicates the orientation of the motif in comparison with the IgH dc-octamer motifs (see Figure 8).

(NF III[340]). The different dc-octamer binding factors may be encoded by a supergene family, similar to the hormone receptors.[227]

There is considerable evidence that Ig gene transcription is also regulated in a negative manner:

1. In myeloma-fibroblast somatic cell hybrids, IgH and Igκ transcription is inhibited,[341,342] and the IgH enhancer is less active than in myeloma cells but as active as in fibroblast cells.[342]
2. Expression in fibroblasts of an introduced rearranged IgH gene is stimulated by the protein synthesis inhibitor, cycloheximide.[343]
3. In transfection experiments in fibroblasts, the specific activity of the IgH enhancer can be increased by either adding an excess of competitor DNA containing the IgH enhancer, or increasing the quantity of DNA transfected.[206,323,342,344]
4. In vitro extracts contain factors which can inhibit transcription.[107,108,345]

These results can be explained by the presence of labile titratable factors in fibroblasts which inhibit the IgH enhancer. A titratable inhibitor(s) probably interacts with the central IgH enhancer (see central-specific inhibitor, Figure 8A).[206,323,342] However, additional fibroblast-specific inhibitors are located on both sides of the enhancer (see IN1 and IN2, Figure 8A),[342] in matrix attachment regions (5′ and 3′ MARs, see Figure 8A),[285] suggesting that matrix attachment may inhibit transcription in fibroblasts. Proteins which bind to IN1 and IN2 have been detected both by genomic footprinting (Figure 8A)[94] and in cell-free extracts (Figure 8A[325,383]). MARs are also located close to Igκ enhancer, suggesting that they may also be involved in regulation of Igκ enhancer activity.[284]

How might the negative trans-acting factors inhibit enhancer activity? In prokaryotes, repressors can sterically prevent binding of positive factors to DNA (see Section III). Induction of the human β-interferon gene may involve dissociation of repressor molecules which sterically block access of constitutive TFs to the promoter (see Reference 97 and bibliography therein, and see Reference 346 for a different view). The steric mechanism may account for inhibition by the centrally located IgH inhibitor (see Figure 8A), but not

for inhibition by IN1 and IN2, which are separated from the IgH enhancer by about 150 bp of DNA (see Figure 8A). Other distantly acting negative regulatory elements have been identified in yeast (for a review, see Reference 347) and in the c-myc[348] and insulin-1[349] promoters, but the mechanism is not clear at present.

Negative regulation is probably a common mechanism of gene control in higher eukaryotes. Protein synthesis inhibitors activate transcription of a number of genes, suggesting that negatively acting labile repressors are common.[350] Extinction of tissue-specific gene expression is observed in various somatic cell hybrids, and there is some evidence that the nonspecific cells contain trans-acting repressors.[351,352] Various promoter elements[353-356] and enhancers[349,357-363] are negatively regulated.

Certain properties of the cell-specific insulin-1 enhancer are strikingly similar to those of the IgH enhancer, including the presence of two different inhibitory sequences[349] and the control of tissue specificity by a combination of both negative regulation in nonpancreatic cells and positive regulation in specific cells.[349,357] The role of the repressors could be to shut down enhancer activity by preventing binding of ubiquitous constitutive factors. This would account for the genomic footprinting result that ubiquitous factors only bind to the IgH enhancer in lymphoid cells.[94]

F. Developmental-Stage-Specific Regulation: The Ig Genes

An early stage in the differentiation pathway of B lymphocytes is the pre-B cell, which has a rearranged heavy- but not light-chain gene and a low level of IgH mRNA. In a later stage, the B cell, the light-chain (kappa or lambda) genes are rearranged as well, but the level of both heavy- and light-chain mRNAs is low. Terminal differentiation to the plasma cell is accompanied by a dramatic increase in both heavy- and light-chain mRNAs (for a review, see Reference 320). Since enhancers are associated with efficient promoters, it was originally thought that through rearrangement the heavy- and light-chain promoters were brought under the influence of the enhancer and thereby activated, and that the presence of large amounts of mRNA in plasma cells could result from efficient transcription due to the presence of strong enhancers.[364] It is now known that this model cannot account for increased expression from the IgH gene. The IgH enhancer and V_H promoters in transfection experiments are as active in pre-B as in plasma cells,[62,365] the transcription rate of the IgH gene is already high in the pre-B cell,[365] and V_H transcription occurs in precursor cells that have not rearranged their IgH genes.[366] These results show that changes in promoter activity cannot account for the large increase in IgH mRNA from the pre-B cell to plasma cell stage, and suggests that posttranscriptional mechanisms play a major role. However, enhancer activation can account to some extent for increased expression from the Igκ gene.

In pre-B cells, the κ loci are unrearranged and transcriptionally inactive. Treatment of pre-B cells with bacterial lipopolysaccharide (LPS) leads to the appearance of DNAase I hypersensitive site at the κ enhancer (Figure 9)[262,263,367,368] and of transcription from pseudopromoters in the κ locus.[263,368,369] The 70Z/3 cell line represents a late pre-B cell in that the Igκ gene is rearranged but transcriptionally inactive.[370,371] LPS stimulation of 70Z/3 cells leads to κ gene transcription. These results suggest that κ gene transcription is activated by the inducible κ enhancer. Two TFs — NF-κE3 and NF-κB — have been identified which bind to the Igκ enhancer (Figure 9).[372] NF-κE3 binds to the Ephrussi E3-like motif, and may be the same factor as NF-μE3 which binds to the E3 motif in the IgH enhancer.[128] This factor is constitutively active in many cell types (see Section IV.E), and is probably not responsible for induction of the enhancer. In contrast, NF-κB is inducible in pre-B cells and could be responsible for the induction of enhancer activity.[129,264] NF-κB is induced by LPS, protein synthesis inhibitors, and phorbol 12-myristate 13 acetate (PMA), an activator of protein kinase C,[129] suggesting that a posttranslational mechanism, possibly phosphorylation, activates NF-κB. This factor is constitutively active in B cells independent of the presence of inducer. However, its continual presence is apparently not essential because the

Igκ gene remains active even in B cells that lack NF-κB.[264] It appears that transient activation of the Igκ enhancer is sufficient for continued transcription of the gene.

Although NF-κB appears to be an important regulator of κ gene expression, its presence is not restricted to B-lymphoid cells. NF-κB is found in a number of cell lines (HeLa, Jurkat) and interacts with other enhancers (SV40-TC motif, cytomegalovirus, human immunodeficiency virus).[129,373] Thus, although NF-κB is used for special purposes in B-lymphocyte differentiation, NF-κB activation probably has broader applications in transcriptional regulation. In addition, the activity of other TFs may be regulated by similar mechanisms to NF-κB because PMA also stimulates the activity of the c-fos,[248-250] SV40,[374] and polyomavirus[375] enhancers.

V. CONCLUSION

Recent improvements in the techniques for the isolation of sequence-specific DNA binding protein (see Section II.C) should lead to the identification, purification, and cloning of the genes of a large number of TFs, and a better understanding of factor structure and mode of interaction with DNA and other TFs. There has already been an advance in the cloning of the genes for several transcription factors, such as TFIIIa and the hormone receptor family, and there are exciting new findings on the structure and functions of these factors (for a review and bibliography, see Reference 227) which are not covered in this review. Slower progress might be expected for TFs which do not bind to DNA, but interact with promoters through other DNA binding proteins. A well-studied example, not discussed here, is the Ad E1a protein.[186] However, an even greater challenge is going to be the study of promoters in their natural locations in chromosomes, and the regulatory mechanisms which are not accurately reproduced by current in vitro and in vivo techniques.

ACKNOWLEDGMENTS

I would like to thank J. L. Imler and C. Schatz for critical reading of the manuscript, J. M. Egly for communicating unpublished results, the secretarial staff for typing the manuscript, C. Werlé and E. Hugonot for artwork, B. Boulay for photography, and the CNRS, INSERM, and ARC (grant no. 6708) for financial assistance.

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