Cloning and Characterization of the Promoter Region of a Gene Encoding a 67-kDa Glycoprotein*

Nabendu Chatterjee‡, Cheng Zou‡, John C. Osterman¶, and Naba K. Gupta‡

From the ‡Department of Chemistry and ¶Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0304

A rat genomic library constructed in λ-EMBL3 (SP6/T7) vector (CLONTECH) was screened using 32P-labeled rat p67 cDNA. A clone containing a segment of 5'-upstream region of p67 genomic DNA was obtained. The DNA (about 1.7 kilobase pairs) was isolated and characterized. Sequence analysis of this DNA fragment showed that the 898 base pairs at the 5'-end of the upstream region was identical to several long interspersed nucleotide sequences. One hundred forty-eight base pairs at the 3'-end contained the beginning of the first exon including the ATG initiator codon. The remaining 652 base pairs in between contained two AT-rich regions and several regulatory sequences. The mRNA initiation site was identified at 89 base pairs upstream from the translation start codon. The DNA fragment was also analyzed by transient transfection. When linked to a firefly luciferase reporter gene, this fragment enhanced transcription in a rat hepatoma cell line (KRC-7). Using a series of deletions in the DNA, the minimum essential promoter region (from −177 to −60) was identified. The promoter activity was also enhanced by treatment with phorbol 13-myristate 12-acetate (PMA). This enhancement required an AP-1 sequence (−298 to −292; 5'-TGACTCA-3') and a similar sequence (−97 to −88; 5'-ATGACATCAT3'). Deletion of either of these sequences significantly reduced PMA enhancement. Deletion of both of these sequences almost completely eliminated PMA enhancement.

Protein synthesis in animal cells is regulated by phosphorylation of a key peptide chain initiation factor, eIF-2.1 Animal cells contain eIF-2 kinases such as heme-regulated inhibitor (KRC-7). The same serum-starved cell line regains p67 transcription after addition of a mitogen phorbol 13-myristate 12-acetate (PMA).

To identify the regulatory sequences in p67 transcription, we have now cloned a segment of the 5'-upstream region of p67 genomic DNA. In the present paper, we describe the characterization of this DNA fragment and identification of the essential promoter region and the PMA-responsive sequences.

EXPERIMENTAL PROCEDURES

Primer

The primers used in different experiments are listed in Table I. The primers were synthesized using the facilities of the DNA Synthesis Laboratory at the University of Nebraska, Lincoln, and Life Technologies, Inc. The preparation of pGEM-p67 cDNA has been described (10).

Preparation of 32P-Labeled Rat p67 cDNA

A 290-bp p67 cDNA fragment was prepared using PCR. The pGEM-p67 cDNA was used as template along with two primers, A and B (Table I). The amplified DNA fragment was then purified and random-labeled using [α-32P]dATP following standard experimental procedures.

Isolation and Sequencing of the 5'-Upstream Region of the p67 Genomic Clone

A rat genomic library constructed in λ-EMBL3 (SP6/T7) vector (CLONTECH) was screened using the 290-bp 32P-labeled rat p67 cDNA. A plaque was identified and later amplified. The phage DNA was isolated from the clone and was digested with BamHI. The digested fragments were analyzed in a Southern blot experiment using a synthetic 70-mer oligonucleotide probe corresponding to −72 to +142 base pairs of p67 cDNA. One 1.7-kb fragment was detected after autoradiography. The DNA fragment was subcloned into the BamHI site of pGEM T7Z(+) vector (Promega) and was sequenced following Sanger's dideoxynucleotide chain termination method (11). The DNA sequence was analyzed using the Genetic Computer Group (GCG) sequence analysis software. This sequence is shown in Fig. 1.

Primer Extension Analysis

The transcription start site of the rat p67 gene was analyzed by primer extension. Total RNA from KRC-7 cells was isolated using the guanidinium thiocyanate method (12). Approximately 10 μg of RNA was used as a template. The primer was an 18-base oligonucleotide (primer C, Table I) corresponding to the inverse complement of +75 to +92 nucleotides of the sequence reported in this paper. The primer (10 pmol) was end-labeled with [γ-32P]ATP and T4 polynucleotide kinase (Promega). The primer extension reaction was carried out with avian myeloblastosis virus reverse transcriptase and Primer Extension System (Promega) following standard procedures. The extended product was analyzed on an 8% sequencing gel and compared with the DNA sequence ladder and 9X-174 HindIII DNA marker.

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† To whom correspondence should be addressed. Tel.: 402-472-2743; Fax: 402-472-9402.

1 The abbreviations used are: eIF-2, eukaryotic initiation factor-2; p67, eIF-2 associated 67-kDa glycoprotein; DNA, deoxyribonucleic acid; bp, base pair(s); kb, kilobase pair(s); PCR, polymerase chain reaction; AP-1, activated protein 1; PMA, phorbol 12-myristate 13-acetate; LINE, long interspersed nucleotide sequence.
Table I

| Primer name | Primer sequence (5′- to 3′-) | Primer position (5′- to 3′-) |
|-------------|------------------------------|----------------------------|
| Primer A<sup>a</sup> | AATAGATGGGAGACCTACC | 1028–1047 |
| Primer B<sup>b</sup> | CAAAGTTTCTATGTAAGCATT | 1296–1318 |
| Primer C<sup>c</sup> | CATGTGGGCGGAGAGGA | +/+/+ |
| Primer D<sup>+</sup> | AGCTATACCTCCTACGCTATAC | −1543 to −1519 |
| Primer E<sup>e</sup> | ACAATTCGACAACTACTGCT | −459 to −440 |
| Primer F<sup>e</sup> | TTGCTCTAGACATTGAGAC | −379 to −360 |
| Primer G<sup>e</sup> | TTGTCAACCGTGTGAGGG | −150 to −171 |
| Primer H<sup>e</sup> | GCCGAAGGCTAATAATA/TTCAATAGAATCCTCACA | −199 to −181/−62 to −43 |
| Primer J<sup>e</sup> | ATGTATCTTGTAAA/TTTATTCTCAGTCACTATA | −48 to −62/−172 to −204 |
| Primer K<sup>e</sup> | GCTCATCCCTGCTCACCTACCTC | +30 to +6 |
| Primer L<sup>e</sup> | ATCACTGAGTTCT/TTAGAGTCCTAAGCAAGA | −344 to −328/−179 to −160 |
| Primer M<sup>e</sup> | AGGTGAGGACTCTA/AAAGCTGTTATGTATGATA | −162 to −179/−328 to −348 |
| Primer N<sup>e</sup> | GCAATATGGGCTGAG/CAGAAAGTTCGTTAATCAATCAG | −492 to −478/−326 to −307 |
| Primer O<sup>e</sup> | TTACGGAATTCCTCTGCTAGCCACATTTGCTTATGGTAT | −312 to −326/−478 to −497 |
| Primer P<sup>e</sup> | TGAACTACATCTACTAATA/TTATATTAGTGGTAGGCAT | −315 to −299/−291 to −272 |
| Primer Q<sup>e</sup> | CCTCAAGCAATAATGAGTTAC/GTGAGTGGTCACAGGA | −275 to −292/−299 to −318 |
| Primer R<sup>e</sup> | CTCTGCTGTTGAAA/AAAAATTGAGCCCAAAGTTG | −114 to −98/−87 to −68 |
| Primer S<sup>e</sup> | GAGGCGATCCTGAGGAGGATG | +34 to +13 |
| Primer T<sup>e</sup> | GAGGCGATCCTGAGGAGGATG | −659 to −639 |
| Primer X<sup>e</sup> | GAGGCGATCCTGAGGAGGATG | −656 to −634 |

<sup>a</sup> Sequences of p67 cDNA (10).
<sup>b</sup> Sequences of reverse complement of the p67 cDNA (10).
<sup>c</sup> Primer used for the primer extension reaction.
<sup>d</sup> Reverse complements of the 5′-upstream region of the p67-genomic DNA (Fig. 1).
<sup>e</sup> Primers used for overlap extension PCR for specific internal deletions.

Cell Culture

The cloned cell-line KRC-7 (a rat hepatoma cell-line; a gift from Dr. J. Koontz, University of Tennessee, Knoxville) was cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 5% (v/v) fetal calf serum, 5% (v/v) calf serum, 100 units/ml penicillin, 50 μg/ml streptomycin, and 10 μM sodium pyruvate at 37 °C with 5% CO<sub>2</sub>.

Preparation of Different Deleted p67 5′-Upstream Region Constructs

Different deleted p67 5′-upstream regions were prepared using PCR and in some cases followed by nested deletions. The DNA sequences were purified and subsequently subcloned into the XhoI and HindIII sites located upstream of the promoterless luciferase gene in the pGL3-basic vector (Promega) to generate the pGL3-deleted p67 5′-upstream region constructs. Table II describes different DNA templates and primer combinations used. Fig. 3 describes the deleted constructs. The preparations of individual constructs are described below.

5′-Deletion—pGL3−477/−326. PCR, pGL3−1531/−24, pGL3−652/−24, pGL3−454/−24, and pGL3−366/−24 were prepared by PCR.

For preparation of pGL3−1531/−24, one forward primer (primer D; Table I) with an XhoI site and a reverse primer (primer H; Table I) with a HindIII site were synthesized. These two primers and the pGEM7-1.7-kb 5′-upstream region template were used to amplify the fragment using Taq DNA polymerase. The PCR-amplified product was purified by Wizard PCR Prep (Promega) and was digested with XhoI and HindIII. The digested fragment was purified and subsequently subcloned into the XhoI and HindIII sites located upstream of the promoterless luciferase gene in the pGL3-basic vector (Promega) to generate the pGL3−1531/−24 construct. The DNA sequence was confirmed by Sanger’s dideoxynucleotide chain termination method (11) using Sequencing II kit (USB).

The experimental procedures for the preparation of pGL3−652/−24, pGL3−454/−24 and pGL3−366/−24 were the same as described above. The primers used were pGL3−652/−24, primer X + primer H; pGL3−454/−24, primer L + primer H and pGL3−366/−24, primer F + primer H.

PCR followed by nested deletion, pGL3−271/−24, pGL3−177/−24 and pGL3−60/−24, pGL3−652/−24 was prepared as described as above (i). This DNA construct was subjected to nested deletion by using the Erase-A-Base system (Promega) and standard procedures.

Promoter in Reverse Orientation—pGL3 reverse −652/−24. Reverse orientation of the promoter in the expression vector was inserted using a HindIII site at the 5′-end and XhoI site at the 3′-end by PCR using primer S and primer T (Table II). During ligation, the promoter was inserted in the reverse orientation of the pGL3 basic vector.

3′-Deletion—pGL3−652/−156. The 3′-deleted construct was generated by PCR. The primers X and G were used (Table II). The amplified fragment was digested, purified, and subcloned.

Internal Deletions—pGL3−180/−60, pGL3A−327/−179 and pGL3−477/−326. The PCR overlap-extension technique of Pease and co-workers (13) was utilized to create the specific internal deletions (Fig. 3, panel D). Initially, two different PCR products were synthesized. For preparation of pGL3−180/−60, the primers were as follows: reaction 1, primer X and primer I (deletion at −60 to −180 nucleotides in reverse orientation, Table I); reaction 2, primer J (deletion at −180 to −60 nucleotides in the forward orientation, Table I) and primer H. The two PCR products were mixed and fused together. The fused products were amplified with primer X and primer H. Details are given in Table II. The amplified DNA was subcloned into pGL3 basic vector.

The procedures for preparation of pGL3−327/−179 and pGL3−477/−326 were the same as described above. Details are given in Table II. In both cases, the fused products were amplified with primer X and primer H. The amplified DNA was subcloned into pGL3 basic vector.

Sequence-specific Deletions—pGL3ΔAP-1 (−298 to −292), pGL3ΔAP-1-like (−97 to −88), and pGL3ΔAP-1 (−298 to −292)ΔAP-1-like (−97 to −88).

Selective deletion(s) of the two putative PMA-responsive sequences were also performed using the overlapping PCR technique as mentioned above. The specific primers (Table I) were designed to eliminate the AP-1 sequence at −298 to −292 or the AP-1-like sequence at −97 to −88 or both (Table II) (Fig. 3, panel E).

Transient Transfection

Approximately, 3 × 10⁴ KRC-7 cells were transiently transfected with different promoter construct by lipopolyamide-mediated transfection (LipofectAMINE™, Life Technologies, Inc.) according to standard procedure. The constitutive expression vector (5 μg of pSV-β galactosidase from Promega) for β-galactosidase was included in the DNA mixture as a marker for transfection efficiency (14). The cells were harvested 48 h after transfection and assayed for luciferase and β-galactosidase activities.

Treatment of Cells for PMA Induction

The cells were transiently transfected with different deletion mutants and were serum-starved by replacing the transfection medium with serum-free medium after 8 h post-transfection. The transfected cells were then treated with 1.5 μM PMA for 2 h before harvesting them.
**Luciferase and β-Galactosidase Assays**

The luciferase activity was measured by the luciferase assay kit (Promega) essentially according to the manufacturer's instructions. The cells were harvested after 48 h post-transfection and lysed in reporter lysis buffer (Promega). Aliquots were used for luciferase and β-galactosidase assays. Cell extracts from untransfected cells and from cells transfected with the pGL3 basic vector alone without the −652/+24 inserted sequence were used as negative controls. Luciferase activities were determined by mixing lysates with luciferase assay buffer containing luciferin, Mg²⁺, and ATP at room temperature and were analyzed immediately by a scintillation counter (15–16). The β-galactosidase activity was analyzed using a commercial enzyme assay system (Promega) at 420 nm. The luciferase activity was normalized with the β-galactosidase activity. The relative luciferase values are the average of three independent experiments. The mean of the luciferase activities relative to pGL3 basic activity ± S.D. are presented in all the figures.

**RESULTS**

**Isolation and Characterization of Rat p67 Genomic Clone**

A total of 2 × 10⁵ plaques from a genomic DNA library were screened using a 290-bp 32P-labeled rat p67 cDNA probe. One positive plaque was isolated after three rounds of rescoring. The phage was amplified in *Escherichia coli* following the standard procedure. The phage DNA was isolated. The DNA was digested with BamHI and analyzed by Southern blotting. A 1.7-kb fragment from the 5'-end of the gene was identified. This fragment was later subcloned into pGEM 7Zf (+) and amplified.

**Sequence Analysis of the 5'-Upstream Region**

The sequence of the 1.7-kb DNA fragment was determined following the Sanger’s dideoxy method (11) (Fig. 1). The 5'-end of the upstream region containing 898 base pairs was identical to several LINE sequences (17–19), and 148 base pairs of the 3'-end contained the beginning of the first exon including the ATG initiation codon. In between these two regions is the proximal promoter of the p67 genome (652 base pairs).

The promoter region contains two potential TATA-like sequences between −40 and −20 and several regulatory sequences (underlined in Fig. 1).

**Determination of Transcriptional Initiation Site by Primer Extension**

The results of a primer extension experiment using total RNA from KRC-7 cells and a 18-base oligonucleotide primer (primer C, Table I) are shown in Fig. 2. A DNA fragment was detected corresponding to a position 89 bases upstream to the ATG codon (Fig. 2, lane 2). No extended signal was observed with yeast tRNA (Fig. 2, lane 1). From the sequence analysis, it was determined that the transcription start site was located about 25 bases downstream from the proximal TATA-like element (Fig. 1).

**Promoter Activity of the 5'-Upstream Region of the p67 Gene**

To determine the promoter region responsible for transcriptional regulation, KRC-7 cells were transiently transfected with the p67 5'-upstream promoter sequence (−1531 to +24 bp) and also different deleted promoter constructs fused upstream to a luciferase reporter gene (Fig. 3). The promoter activities were then determined by analysis of luciferase expression in the transfected cell extracts. The luciferase activity was normalized with the β-galactosidase activity in the same cell extracts. The normalized luciferase expressions in cells transfected with different pGL3 constructs (+ promoter) and pGL3 basic (− promoter) were compared. The fold increase (× fold) with pGL3 constructs (+ promoter) over the pGL3 basic (− promoter) is presented in different figures.

| Template | Primes | Modified p67 5'-upstream region |
|----------|--------|-------------------------------|
| A, 5'-Sequential deletions | Primer D + primer H | −1531/+24 |
| pGEM7-1.7-kb DNA | Primer X + primer H | −652/+24 |
| pGEM7-1.7-kb DNA | Primer E + primer H | −454/+24 |
| pGEM7-1.7-kb DNA | Primer F + primer H | −369/+24 |
| B, Reverse orientation | Primer S + primer T | Reverse −652/+24 |
| C, 3'-deletion | Primer X + primer G | −652/-156 |
| D, Internal deletions | (Primer X + primer I) + (primer J + primer H) | Δ −180/-60 |
| pGEM7-1.7-kb DNA | (Primer X + primer K) + (primer L + primer H) | Δ −327/-179 |
| pGEM7-1.7-kb DNA | (Primer X + primer M) + (primer N + primer H) | Δ −477/-326 |
| E, Sequence-specific deletions | (Primer X + primer O) + (primer P + primer H) | ΔAPI1(−298/−292) |
| pGEM7-1.7-kb DNA | (Primer X + primer Q) + (primer R + primer H) | ΔAPI1-like(−97/−88) |
| pGEM7-1.7-kb DNA | (Primer X + primer O) + (primer P + primer H) | ΔAPI1−298/−292 |
| pGEM7-1.7-kb DNA | AP-1-like(−97/−88) | ΔAPI1−298/−292 |

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The nucleotide sequence of a 1.7-kb DNA containing a segment of the 5'-upstream region of the rat p67 gene. The nucleotide sequences were determined following Sanger’s dideoxy method (11). The numbers represent the nucleotide position relative to the transcription initiation site (+1). The translation initiation codon ATG is presented in bold and at position +89. The LINE sequences are boxed. The putative DNA binding sequences like AP-1, AP-3, SP-1, heat-shock element and others are underlined. The purine-rich direct repeat is indicated by the double underline. This sequence has been submitted to GenBank with accession number U37710.

Identification of the transcription initiation site of the rat p67 gene using primer extension analysis. The end-labeled 18-nucleotide primer C (Table I) was hybridized at 65 °C to 10 μg of yeast tRNA (lane 1) or total RNA from KRC-7 cells (lane 2) and then extended using reverse transcriptase at 42 °C for 60 min. The primer extended product was run on an 8% sequencing gel alongside a dideoxy sequencing reaction of the p67 genomic clone. As the sequencing primer was the same as that of the primer extension reaction, the antisense strand is shown. Exposure time was 18 h with intensifying screens.

Functional Analysis of the p67 Promoter Using Internal Deletions—To further define the cis regulatory regions, internal deletions were performed (Fig. 3, panel B). The luciferase expression from cell lysates transfected with pGL3Δ−180/−60 suggests that the essential promoter region of the p67 gene lies between −180 and −60 (panel C). This result also correlates with the previous result in panel B. The luciferase expression was drastically reduced when transfected with pGL3Δ−180/−60. These results, in agreement with the results shown in Fig. 4 (panel B, 4th and 5th bars), suggest that the essential promoter region lies between −180 and −60. As shown in panel C, the luciferase expression was not significantly reduced when transfected with pGL3Δ−180/−60. The promoter activity was significantly reduced upon serum starvation (panel B, 5th bar). However, addition of PMA to these serum-starved cells significantly enhanced (∼50-fold) p67 promoter activity (panel B, 5th and 6th bars).

Identification of the PMA-responsive Sequences in the p67 Promoter

Initially, we used different p67 constructs described in Fig. 3 and determined the promoter activity with or without PMA. In several cases examined (Fig. 6, panels A–C), the results were qualitatively the same. However, as shown in the absence of PMA, the luciferase expression in pGL3Δ−652/−156 (panel B, 11th bar) and pGL3Δ−180/−60 (panel C, 1st bar) were reduced.
to less than 25% maximum expression (panel A, 5th bar). In the presence of PMA, the luciferase expression remained essentially the same in pGL3-652/124 (panel B, 12th bar) but increased significantly in pGL3-D2180/260 (panel C, 2nd bar). This construct (pGL3-D2180/260) contains the AT-rich region not present in pGL3-652/124. These results suggest that (i) the AT-rich region in pGL3-D2180/260 and (ii) other possible PMA-responsive sequences in this construct (pGL3-D2180/260) are necessary for PMA induction.

To identify the PMA-responsive sequences, we prepared different constructs deleting specific nucleotide sequences. Fig. 3 describes the preparations of these such constructs with deletions at an AP-1 (−298 to −292) and an AP-10-like (−97 to −88) element. Fig. 7 shows the effects of such deletions on luciferase expression. The wild type promoter (pGL3-652/24) was used as a control. Upon PMA addition, this promoter enhanced luciferase expression approximately 6-fold (5th and 6th bars). Deletions at the AP-1 element (−298 to −292) (7th and 8th bars) and the AP-1-like element (−97 to −88) (9th and 10th bars) significantly reduced luciferase expression. When both of these sequences were deleted, PMA enhancement of luciferase expression was almost totally eliminated (11th and 12th bars).

**DISCUSSION**

In this paper, we describe cloning and characterization of a 1.7-kb DNA fragment containing a segment of the 5′-upstream region of the p67 gene. Some significant observations are as follows.

The 3′-end of the DNA fragment is part of the first exon. The transcription start site was located 89 bases upstream from the initiator codon and was marked as +1. No classical TATA element was detected at the expected position. Two AT-rich regions were present between −40 and −20. Similar AT-rich regions are also present in c-jun (21). In several cases reported (22–24), these AT-rich regions function similarly like TATA sequence. The 5′-upstream region contains LINE sequence from −1549 to −652. The LINEs are defined as a major family of long interspersed nucleotide elements present in the genome of humans, primates, and rodents. The functions of these LINEs (if any) in gene expression are not known.

The 652 bp between the end of the LINE sequence and the
transcription start site is the promoter region of the p67 gene. A DNA sequence containing this promoter region (−652 to +24) was inserted upstream of the promoterless luciferase gene in the pGL3 basic vector. When transfected into confluent KRC-7 cells, this construct produced an 11-fold increased luciferase expression over that observed with the pGL3 basic vector. This promoter region contains multiple cis-acting elements. Some of these elements that differ by no more than 1 base pair from the consensus sequences of known regulatory elements are listed as follows: Ets-like element (20) at −645 to −639; SP-1-like elements (20, 25) at −609 to −600 and at −486 to −478; Oct-like element (20) at −500 to −493; CArG-like element (26) at −410 to −401, heat-shock element (27) at −326 to −310, AP-1-like element (20) at −298 to −292; AP-3 element (20) at −143 to −132; and AP-1-like sequence (21) at −97 to −88. Using different deletion mutants, we mapped the minimum essential region located between −177 and −60. A purine-rich direct repeat was located at −167 to −144 in the essential promoter region. This sequence includes an 11-base pair direct repeat of 5′-AACARAAGAA-3′ (R = purine). The relevance of this region, at present, is unclear. In human c-FOS gene, an 8-base pair direct repeat is apparently important in promoting the basal level of expression of the gene. This sequence is not required for PMA induction (28). In addition to the direct repeat, this essential region contain one AP-1-like sequence (−97 to −88) and an AP-3-like sequence (−142 to −132).

Although the essential promoter region is necessary for basal transcription, other regulatory elements may also be used to induce transcription under different physiological conditions. In this work, we observed that addition of a mitogen, PMA, to the serum-starved KRC-7 cells increased transcription by 50-fold. Using different deletion mutations, we provide evidence that an AP-1 sequence (−298 to −292) and one AP-1-like sequence (−97 to −88) are necessary for this induced transcription. The functional assays indicate that the AP-1-like element at −97 confers the strongest response to PMA and the classical AP-1 is next. However, to achieve maximal PMA response, both the elements must be present. These results suggest cooperation among the PMA-responsive elements.

PMA also induces expression of a number of cellular genes (29–31). It is not clear whether different genes use common or distinct elements. Several cellular proteins such as the AP-1 (32–34), NF-κB (35–36), or novel nuclear factors (37) have been implicated in this induction. In our studies, we observed high levels of PMA induction in KRC-7 cells transfected with pGL3−652 construct only in serum-starved cells. We did not observe significant PMA induction in confluent cells. It is not clear whether this difference is due to the presence of a labile inhibitor (38) or the presence of CArG element that may act as a repressor in the presence of serum (39).

Several promoters for translational initiation factors eIF-2α (40), eIF-4A (41), and eIF-4E (42) have been reported. eIF-4E promoter has several c-myc regulatory elements (42) and eIF-2α has one (40). Both these genes are regulated by c-myc (43). eIF-4E gene also has a potential p53-binding element (42). The p67 promoter does not contain either c-myc or p53-binding element. On the other hand both p67 promoter (Fig. 1) and eIF-2α promoter contain heat shock element. A past report has indicated that eIF-2α is a heat shock protein (44). Recent work in our laboratory has indicated that p67 level in the cell is also significantly increased upon heat shock.2 Another interesting difference is that whereas the p67 promoter has only 35% G + C, the other initiation factor promoters contain at least 52% G + C (40–42, 45). Also, the p67 promoter has two AT-rich regions. This promoter lacks the CAAT element. The eIF-4A promoter has one classical TATA and CAAT elements. These sequences are absent in eIF-2α or eIF-4E promoters. Another interesting difference is that whereas p67 gene contains a single transcription start site, eIF-2α or eIF-4A contains multiple transcription start sites.

2 M. Chatterjee, unpublished observations.
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