A 69-Base Pair Fragment Derived from Human Transcobalamin II Promoter Is Sufficient for High Bidirectional Activity in the Absence of a TATA Box and an Initiator Element in Transfected Cells

ROLE OF AN E BOX IN TRANSCRIPTIONAL ACTIVITY

(Received for publication, June 15, 1998, and in revised form, August 17, 1998)

Ning Li‡ and Bellur Seetharam‡§§

From the Division of Gastroenterology and Hepatology, Department of ‡Medicine and §Biochemistry, Medical College of Wisconsin and Veterans Medical Center, Milwaukee, Wisconsin 53226

A 69-base pair (bp) (−581/−513) fragment derived from human transcobalamin II distal promoter constructed upstream of a chloramphenicol acetyltransferase reporter gene demonstrated high bidirectional promoter activity in transfected epithelial Caco-2 cells. DNase I footprinting, gel mobility shift, supershift, and mutagenesis studies with the 69-bp fragment demonstrated that a GC box (−588/−559) and an E box (−523/−528), which interacted with Sp1/Sp3 and USF1/USF2 (where USF is upstream stimulatory factor), respectively, were required for the full transcriptional activity of this fragment. Whereas mutations in the GC box reduced the promoter activity by 50%, mutations in the E box alone or in both the E box and GC box resulted in 90% loss of transcriptional activity. The essential role of the E box in the bidirectional promoter activity was further demonstrated by transient transfection in Caco-2, K-562, and HeLa cells using a 29-bp (−541/−513) fragment that contained only the E box. Based on these results we suggest that 1) the E box is essential for both the GC box-dependent and -independent promoter activity of the 69-bp fragment, 2) cooperative interactions between Sp1/Sp3 and USFs are required for the full activation of the 69-bp promoter activity, and 3) the single E box is able to mediate bidirectional transcription in transfected cells in the absence of an obvious TATA box or a known initiator element.

Eukaryotic gene expression is controlled by a combination of effects from various cis-elements present in the promoter and enhancer regions. One such cis-element is the E box (CANNTG), which is recognized by a variety of basic helix-loop-helix (bHLH) transcription factors. Two types of E box, depending upon the nature of the two central nucleotides, are described (1). Whereas the E box with the sequence CACGTG is referred to as class A, the E box with the sequence CAGCTG belongs to class B. The functions of the E box are very divergent and to a large extent dependent upon the transcription factors that bind to it. These include neurogenesis, myogenesis, sex determination, T-cell/B-cell, and pancreatic specific gene expression, as well as cell proliferation and differentiation (reviewed in Ref. 2). The selective binding mechanism by which multiple factors bind to the same target site (class A or B) is not clear. It may involve competition among the factors influenced by sequences flanking the consensus CANNTG or interactions with other proteins binding to adjacent sites (3).

Upstream stimulatory factor (USF) is a ubiquitous transcription factor (4) belonging to the class B proteins that also include Myc (5), Max (6), Mad (7), Mxi (8), TFE3 (9), TFE4 (10), and TFEC (11). USF was first identified for its stimulation of transcription from the adenovirus late promoter (12) and was purified from HeLa cells as two polypeptides, USF1 (43 kDa) and USF2 (44 kDa) (14). USF1 and USF2 bind to the E box (CACGTG) as homo- and heterodimers, and their ratios vary in different cell types (4). Although the exact biological roles of USF1 and USF2 are not fully understood, it has been reported that they play a critical role in both the basal (15–19) and signal-induced (4, 20–22) expression of cellular genes.

Previously (23) we have shown that the promoter activity of human transcobalamin II (TC II), a plasma transporter of cobalamin (24), is relatively weak and controlled positively by a distal GC box and negatively by a proximal GC/GT overlapping box. In the present study, we have identified a 69-bp sequence (−581/−513) from the distal region of the TC II promoter (25) that did not contain an obvious TATA box or known Inr element but possessed a high promoter activity in transfected cells in an orientation-independent manner. The bidirectional promoter activity was due to interactions between Sp1/Sp3 and USF1/USF2 that bound to GC box and E box, respectively. Furthermore, a 29-bp sequence (−541/−513) containing only the E box was sufficient by itself to mediate transcription in the absence of other cis-elements. This finding suggests that TATA-/Inr− promoters may use an E box as a core element to direct basal transcription.

MATERIALS AND METHODS

Construction of Promoter-CAT Reporter Plasmids—Promoterless plasmid, pCAT-Basic (pCAT-B) (Promega, Madison, WI), was used for the preparation of the CAT fusion reporter constructs. Various truncated TC II-promoter fragments were generated by polymerase chain reaction (PCR) and inserted into the pCAT-B vector at a PstI site upstream from the CAT gene. A total of seven promoter fragments were amplified, and the sequences of each pair of primers used for PCR are shown in Table I. The DNA sequence of each of the promoter fragments

28170 This paper is available on line at http://www.jbc.org
TABLE I

| Promoter Fragments | Forward Primer | Reverse Primer |
|-------------------|---------------|---------------|
| F1(-1014/-679)    | 5' ACCTCCTGGGTCAAGGATCC | 5' CGCGCTGGCTGACAGCAGC |
| F2(-746/-513)     | 5' CTCCAGGGACTCTCCCACT | 5' CTGCCAGGGTCGAGCAGC |
| F3(-512/-158)     | 5' CTGCGAGTCTTCACCTTTG | 5' CTGCCAGGGTGCGCAGG |
| F4(-163/+185)     | 5' CTGCGAGTCTTCACCTTTG | 5' GTGCCAGCTAGCCAACCTG |
| A(-746/+689)      | 5' CTCCAGGGACTCTCCCACT | 5' CGCGCTGGCTGACAGCAGC |
| B(-688/-570)      | 5' GGCTGAGTGCGAGCCAGG | 5' CTGAGTTGCGGAGGAGGAT |
| C(-581/-513)      | 5' CAAAATTCCTGACAGCCCAACTGA | 5' CTGCGAGTCTTCACCTTTG |

Electrophoretic Mobility Shift Assay (EMSA)—The promoter fragment C (-581/-513) was labeled with [32P]dCTP using the Klenow fragment of DNA polymerase. DS oligonucleotide CI or CII (Table II) was labeled at the 5' termini with [γ-32P]ATP using T4 polynucleotide kinase. Labeled probe (–2 × 10^4 cpm) was incubated for 15 min at 22 °C with 2.2 μg of nuclear extract in 10 μl of reaction buffer (10 mM Tris-HCl, pH 7.5, 4% glycerol, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 mM NaCl, and 50 μg/ml poly(dI-dC)) for 5 min in preincubation with the probe. For immunosupershift assay, the nuclear extract was preincubated with 1.5 μg of affinity purified rabbit polyclonal antibody against USF1, USF2, or c-Myc (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) at 22 °C for 30 min prior to incubation with the probe. The reaction mixture was then subjected to 4% polyacrylamide gel electrophoresis in 0.5× TBE buffer (44.5 mM Tris-HCl, 44.5 mM boric acid and 1 mM EDTA) at 100 V. The protein-DNA complexes were visualized by autoradiography.

RESULTS

A 69-bp Sequence Derived from the 5' Region of the TC II Gene Activates Transcription in an Orientation-independent but Position-dependent Manner—Our previous 5'-deletion studies (23) have demonstrated that the transcriptional activity of the TC II-promoter fragments, including the longest (–1014 to +34), is very weak in Caco-2 cells. To identify regions that may potentially have higher promoter activity, four pro-
Role of E Box in Minimal Promoter Transcriptional Activity

**TABLE II**

DNA sequence of oligonucleotides used in EMSA

| Oligo | Sequence | GC box | Myb |
|-------|----------|--------|-----|
| Sp1 (Promega) | 5’ ATTGCATGGGGCAGGCGGGCGAG 3’ | -581 | |
| Cl (Wild-type) | 5’ GCCAACAAGTCAGGGGAAGCCTGTGCTG | -583 | |
| Cl-M (Mutated) | 5’ GCCAACAAGTCAGGGGAACCTGTGCTG | **| **|
| Myb | 5’ CCGCACAAACTGAAGGTctagatt | -541 | |
| CII (Wild-type) | 5’ CAGCAATGCTTGCTGACACATGCAG | -513 | |
| CII-M (Mutated) | 5’ CAGCAATGCTTGAGCGAGCACTGCAG | **| **|

- A mismatched nucleotide for consensus sequence of GC box is double underlined.
- Mutated nucleotides are indicated by asterisks.
- The sequence indicated by the lowercase letters in the oligo-Myb contains a restriction enzyme XhoI site which is not present in the 5′-flanking region of TCII gene.
- The E box (CACGTG) within the 14-bp palindrome is underlined.

Promoter fragments (F1–F4) generated by PCR were cloned into a promoterless CAT reporter vector, pCAT-B. Upon transient transfection in Caco-2 cells (Fig. 1A), only the fragment F2 (−746/−513) possessed promoter activity above the background activity produced by the promoterless pCAT-B vector. The promoter activity of this fragment (20-fold) was orientation-independent (Fig. 1B) as the fragment F2 in a reverse orientation (−513/−746) revealed a similar level (19-fold) of transcriptional activity.

In order to identify the elements within the 234-bp fragment that were responsible for its high bidirectional transcriptional activity, this fragment was further dissected into three regions (A, B, and C) (Fig. 1B). The 5′-terminal region A (58 bp, −746/−689), which contained an inverted CCAAT element and a previously described GC box, and an E box (CACGTG)—a palindrome (Fig. 2A). To identify functional cis-elements in the 69-bp region, DNase I footprinting analysis was performed using nuclear extracts from Caco-2 and HeLa cells. Two protected regions, CI and CII, were revealed using both cellular nuclear extracts (Fig. 2A). Region CI (−578/−559) included a Myb-binding site and a previously described GC box, and region CII (−532/−519) contained a 14-bp palindromic sequence with an E box (CACGTG) of class B in the center of the palindrome (Fig. 2B).

Nuclear proteins binding to the two protected regions were then examined by EMSA using nuclear extracts from Caco-2 cells and the probes covering the two regions, respectively. Two protein-DNA complexes were revealed when DS oligonucleotide CI was used as a probe (Fig. 3A, lane 2). Both complexes were eliminated when competition was carried out using unlabelled oligonucleotide Sp1 containing the consensus sequence of the GC box (lane 3) or the wild-type oligonucleotide CI sequence (lane 4). The formation of the two complexes was not affected when the oligonucleotide CI-M which contained point mutations in the GC box (Table II) or the oligonucleotide Myb was used as competitors (lanes 5 and 6). These results indi-
cated that the binding of the nuclear proteins in region CI occurred specifically at the GC box rather than at the Myb site. Supershift analysis using antibody against Sp1 and Sp3 has indicated that the complex I was formed by interaction with both Sp1 and Sp3, and the complex II was formed by interaction with Sp3 alone (23).

Gel shift analysis using oligonucleotide CII as a probe also showed the formation of two complexes (Fig. 3B). However, one of the complexes was very dominant whereas the other was very faint (lane 2). Both complexes were abolished when competed with excess of unlabeled wild-type oligonucleotide CII (lane 3) but not with oligonucleotide CII-M (lane 4) in which the 14-bp palindromic sequence was mutated including the core sequence of the E box (Table II).

Both the GC Box and the E Box Are Required for the Transcriptional Activity of the 69-bp Promoter Region—To evaluate the role of the GC box and the E box on the promoter activity of the 69-bp region, the same mutations that eliminated nuclear protein binding (Fig. 3) were introduced into the plasmid construct C(-581/-513). Fig. 4 shows the CAT activity of the wild-type or the mutant constructs transfected in Caco-2 cells. Mutations in the GC box resulted in about 50% loss of the CAT activity of the 69-bp fragment, whereas mutations in the E box alone or in both the GC box and the E box diminished the transcriptional activity by about 90%. These results indicated that both the GC box and the E box were required for the full transcriptional activation of the 69-bp fragment. However, the relative contributions of these two cis-elements were different. The presence of the E box was essential for the transcriptional activity contributed by the GC box but not the other way around. On the other hand, the presence of the GC box enhanced the transcriptional activity of the E box by 2-fold, suggesting that there could be functional physical interactions between the nuclear proteins that bind to the two sites. In
order to test this possibility, gel-shift analysis was carried out using the 69-bp fragment containing both the GC box and the E box.

Nuclear Proteins Bound at the GC Box and the E Box Interact Physically—When the labeled 69-bp promoter fragment was allowed to bind to the nuclear extracts from Caco-2 cells, at least five DNA-protein complexes were revealed (Fig. 5, lane 1). When competed with excess unlabeled oligonucleotide CI (lane 3) or Sp1 (lane 6) which contained the GC box, the complexes I, II, III, and IV were eliminated. When the competition was carried out using unlabeled oligonucleotide CI-M containing mutations in the GC box (lane 2) or CII-M containing mutations in the E box (lane 5) did not affect the formation of the complexes I–V. These results suggested that the formation of the complexes I and II was the result of physical interactions between nuclear proteins bound to both the GC box and the E box since both the complexes were unable to form in the presence of either unlabeled oligonucleotide CI (lane 3) or CII (lane 4).

Since the 69-bp fragment containing the wild-type E box was essential for driving transcription in Caco-2 cells (Fig. 4), additional studies were carried out using a 29-bp fragment, which corresponded to the footprinting region CII containing the E box, to test whether it is able to drive transcription not only in Caco-2 cells but in other cells as well.

The Oligonucleotide CII (29-bp) Is Able to Direct Bidirectional Transcription in Three Types of Cells—Initially we tested by EMSA whether the 29-bp fragment is able to bind to nuclear factors in K-562 and HeLa cells. As shown in Fig. 6, two DNA-protein complexes were formed when the 32P-labeled CII fragment was incubated with nuclear extracts from either HeLa (Fig. 6A, lane 1) or K-562 (Fig. 6B, lane 1) cells. This pattern was similar to that obtained using nuclear extracts from Caco-2 cells (Fig. 3B). The formation of the two complexes were eliminated when competed with excess unlabeled oligonucleotide CII (Fig. 6, A and B, lanes 2–4). In contrast, the oligonucleotide CII-M was unable to compete for the formation of the two complexes (Fig. 6, A and B, lanes 5–7). These results suggested that nature of the nuclear proteins binding to the 29-bp was similar or identical in all the three cell lines tested and that the binding was specific to the E box.

The ability of the 29-bp fragment to drive transcription was then tested in Caco-2, HeLa, and K-562 cells. As shown in Table III, the 29-bp fragment was able to drive CAT gene transcription in both orientations in all the three cell lines tested (Table III), and the CAT activity noted, 8–11-fold, was similar to that of the SV40 promoter. Since the E box (CACGTG) can interact with multiple transcription factors of similar or identical in all the three cell lines tested and that the binding was specific to the E box.

USF Binds to the E box—At least nine members of the bHLH/LZ family of transcription factors bind either as hetero- or homodimers to the E box core sequence (CACGTG). Whereas the c-Myc, Max, Mad, and Mxi1 are involved in the Myc network in controlling cell proliferation and differentiation (32), the others, TFEB, TFE3, and TFEC belong to the Mit subfamily (33) and are involved in the regulation of immunoglobulin heavy chain and insulin gene expression, and USF which is ubiquitously expressed (4) stimulates transcription of many genes (15–22). Thus, we chose USF and c-Myc as the first candidates to examine their binding to the E box.

As shown in Fig. 7 similar EMSA pattern was obtained using nuclear extract from both Caco-2 (Fig. 7A) and HeLa (Fig. 7B) cells. When the nuclear extracts were incubated with anti-USF1 antibody (Fig. 7, A and B, lane 2), the complex I was...
diminished and the complex II was completely abolished, indicating that both complexes contained USF1. When the nuclear extract was incubated with anti-USF2 antibody (Fig. 7, A and B, lane 3), formation of the complex I was mainly interrupted. This result indicated that complex I represented a combination of both USF1 and USF2. Addition of both anti-USF1 and anti-USF2 antibodies to the nuclear extracts resulted in complete abolishment of the complex I and II (Fig. 7, A and B, lane 4). In contrast, antibody against c-Myc did not affect the formation of the two complexes (Fig. 7, A and B, lane 5).

**DISCUSSION**

The present study has provided some insights into the basal and activator-dependent transcription using a human TC II promoter fragment that lacked both a TATA box and an Inr element. By deletion and transient transfection studies, we have demonstrated that a 69-bp DNA sequence from the human TC II promoter possesses bidirectional promoter activity but not an enhancer activity (Fig. 1B, fragment C). The 69-bp fragment did not contain an obvious TATA box or a known Inr element but contained a GC box with one mismatch and a 14-bp palindromic sequence (TGCTCACGTGACCA) with an E box (underlined) in its center. These observations raised an interesting issue as to how the 69-bp DNA fragment without a TATA box and Inr element functioned as a promoter.

**TABLE III**

| Plasmid constructs | Relative CAT activity |
|--------------------|----------------------|
|                    | Caco-2 | HeLa | K-562 |
| CII-S(−541/−513)-P | 8.5 ± 0.8 | 9.2 ± 0.6 | 11.0 ± 1.3 |
| CII-AS(−513/−541)-P | 8.9 ± 0.9 | 9.5 ± 1.0 | 11.6 ± 1.5 |

Our EMSA experiment (Fig. 3) has shown that both the GC box and the E box were functional in binding to nuclear factors, and immuno supershift analysis further demonstrated that the GC box interacted with Sp1 and Sp3 (Fig. 3, Ref. 23), whereas the E box was recognized by both USF1 and USF2 (Fig. 7). Site-directed mutagenesis (Fig. 4) demonstrated that both the GC and the E box were required for the full promoter activity of the 69-bp fragment. However, their individual contribution toward the promoter activity was not equivalent. Mutations in the GC box reduced the promoter activity by 50%, whereas mutations in the E box alone or in both the E box and the GC box did not affect the formation of the two complexes (Fig. 7, A and B, lane 5).
box resulted in about 90% reduction of the promoter activity. These results implied that the E box was required for both the GC-dependent and -independent promoter activity of the 69-bp fragment, and there was potential cooperative interactions between nuclear factors that bound to these two cis-elements. Direct evidence for physical interactions between Sp1 or Sp3 and USFs was provided by EMSA (Fig. 5). The essential role of the E box in mediating transcription was further demonstrated by transfection of the fusion plasmid containing the DS oligonucleotide II (29-bp) which contained only the E box in three different cell lines (Table III).

The observation that a short (29-bp) DNA fragment containing only one recognizable cis-element, an E box, is sufficient to direct bidirectional transcription efficiently is very interesting. In general, a eukaryotic core promoter contains either a TATA box or an Inr element that are recognized by the TATA-binding protein (TBP), a component of the TFIIID complex, and Inr-binding protein, respectively. TBP or Inr-binding protein plays a central role in recruiting basal transcription factors and RNA polymerase II forming a preinitiation complex (PIC). However, there are a few reports that have demonstrated that an activator-binding site alone is sufficient for a minimal promoter activity in transfected cells. These include the glucocorticoid response element (34) and the Ets motif (35–37). The mechanism by which the 29-bp sequence functions as a minimal promoter is not known. It is possible that USF bound to the E box stabilized the binding of TFIIID to a cryptic TATA element through protein-protein interactions. Alternatively or additionally, USF bound to the E box can recruit TFIIID and/or other components of the basal transcription machinery to the promoter and facilitate the assembly of the PIC. Several lines of evidence support this possibility. First, it has been reported (38, 39) that TBP is capable of binding to a number of sequences that are completely unrelated to the consensus sequence of the TATA element. Second, USF has been shown to be able to interact with TFIIID (12, 40), increase the rate or stability of TFIIID binding (41), and stabilize formation of the PIC (42). Third, USF is also able to interact with other transcription factors, including TBP associated factor TAFII55 (43), transcriptional cofactor PC5 (44), and TFII-I (45, 46) which can bind to both the Inr and the E box.

Another interesting aspect of this study is the distinct roles of the E box and the GC box in the transcriptional activity of the 69-bp sequence. Although the presence of the E box was essential for the transcriptional activity due to the GC box, the presence of the GC box was not required for the transcriptional activity due to the E box. This observation implied that USF mainly played a role in transcriptional initiation while the Sp1 stimulated the transcription. This observation is somewhat surprising since it has been shown (47–49) before that Sp1-binding site could direct transcriptional initiation of several TATA-less promoters. Our finding that USF-binding site was more efficient than that of Sp1-binding site in mediating transcriptional initiation could be a general phenomenon or restricted to a specific context of a promoter or cells. Nevertheless, our finding is in agreement with the hypothesis (50) that USF1 may not directly be responsible for transcription but functions via interactions with other proteins. In this regard, it is interesting to note that USF1 does not only bind to the E box but also to the pyrimidine-rich Inr element (45, 46). In addition, ectopically expressed USF1 could stimulate transcription initiation through Inr element (46). Therefore, it is likely that the E box functions similarly to that of the Inr element if it is located near the transcriptional start site. Our finding that both the 69-bp (Fig. 1B) and the 29-bp sequence (data not shown) mediated transcription in a position-dependent manner also supports a recruiting rather than an activating function of the USF bound to the E box. Indeed, USF-binding site near the transcriptional start site has been found being essential for the basal promoter activity of some TATA-less promoters (15, 18, 19).

The functional significance of the role of the E box in TC II transcription is not known. Although the binding of USF to the E box may not affect the basal transcription of TC II in vivo due to its distal location from the start sites of the TC II gene, the potential binding in vivo of other members of bHLH/LZ family cannot be ruled out. Some of these could include Myc/Max, Mad/Max, and Max/Max. Elevation of plasma TC II levels is noted in a variety of cancers (51), and our hypothesis at the present time is that the E box by binding to Myc family may increase the transcription of the TC II gene which in turn will result in increased secretion of TC II to the circulation. Future studies with ectopic expression of the proteins belonging to the Myc family will address the role of these transcription factors in the in vivo transcription of the TC II gene.

In summary, we have identified a 69-bp DNA fragment containing a GC box and E box that is able to mediate transcription in vivo in an orientation-independent manner. The transcriptional activity was due to interplay between Sp1/Sp3 and USF1/USF2 that bind to these two cis-elements. Moreover, 29-bp fragment localized to the 3'-end of the 69-bp fragment containing only the E box was sufficient by itself to drive transcription, implying that USF bound to an E box can initiate and activate transcription in the absence of TATA box or other known Inr elements.

REFERENCES
1. Dang, C. V., Dolde, C., Gillison, M. L., and Kato, G. J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 599–602
2. Murre, C., Brain, G., van Dijk, M. A., Engle, I., Furnari, B. A., Massai, M. E., Matthews, J. R., Quong, M. W., Rivera, R. R., and Stuiver, M. H. (1994) Biochem. Biophys. Res. Commun. 211, 128–135
3. Bendall, A. J., and Molloy, P. L. (1994) Nucleic Acids Res. 22, 2801–2810
4. Sirito, M., Lin, Q., Maity, T., and Sawadogo, M. (1994) Nucleic Acids Res. 22, 457–463
5. Blackwell, T. K., Kretzner, L., Blackwood, E. M., Eisenman, R. N., and Weintraub, H. (1990) Cell 60, 1151–1161
6. Berezney, R., and Eisenman, R. N. (1991) Science 251, 1211–1217
7. Ayer, D. E., Kretzner, L., and Eisenman R. N. (1993) Cell 72, 221–226
8. Zervos, A. S., Gyuris, J., and Brent, R. (1993) Cell 72, 223–232
9. Carr, C. S., and Sharp, P. A. (1990) Mol. Cell. Biol. 10, 4384–4388
10. Beckman, H., Su, L.-K., and Radesch, T. (1996) Genes Dev. 4, 167–179
11. Zhao, G.-Q., Zhao, Q., Zhou, X., Mattei, M.-G., and de Crombrugghe, B. (1993) Mol. Cell. Biol. 13, 4505–4512
12. Sawadogo, M., and Roeder, R. G. (1985) Cell 43, 165–175
Role of E Box in Minimal Promoter Transcriptional Activity

28177

13. Carthew, R. W., Chodosh, L. A., and Sharp, P. A. (1985) Cell 43, 439–448
14. Sawadogo, M., van Dyke, M. W., Gregor, P. D., and Roeder, R. G. (1988) J. Biol. Chem. 263, 11985–11993
15. Outram, S. V., and Owen, M. J. (1994) J. Biol. Chem. 269, 26525–26530
16. Vander Zee, C.-A., Jordan, E. M., and Breen, G. A. M. (1994) J. Biol. Chem. 269, 26525–26530
17. Morris, J. K., and Richards, J. S. (1996) J. Biol. Chem. 271, 6972–6977
18. Ikeda, K., Inoue, S., Orimo, A., Sano, M., Watanabe, T., Tsutsumi, K., and Muramatsu, M. (1997) Biochem. Biophys. Res. Commun. 236, 765–771
19. Ebra, S., Kawasaki, S., Nakamura, I., Tsutsumimoto, T., Nakayama, K., Nikaido, T., and Takaota, K. (1997) Biochem. Biophys. Res. Commun. 240, 136–141
20. Shih, H., and Towle, H. C. (1994) J. Biol. Chem. 269, 9380–9387
21. Lefrancois-Martinez, A.-M., Martinez, A., Antoine, B., Raymondjean, M., and Kahn, A. (1995) J. Biol. Chem. 270, 2640–2643
22. Wang, D., and Sul, H. S. (1995) J. Biol. Chem. 270, 28716–28722
23. Li, N., Seetharam, S., and Seetharam, B. (1995) Biochem. Biophys. Res. Commun. 280, 756–764
24. Graham, F. L., and Van der Eb, A. J. (1973) Virology 52, 456–467
25. Herbold, P., Bourachot, B., and Yaniv, M. (1984) Cell 39, 653–662
26. German, C. M., Moffat, L. F., and Howard, B. H. (1982) Mol. Cell. Biol. 2, 1044–1051
27. Seed, B., and Sheen, J.-Y. (1988) Gene (Amst.) 77, 271–277
28. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
29. Bradford, M. M. (1976) Anal. Biochem. 112, 195–203
30. Ryan, K. M., and Birnie, G. D. (1996) Biochem. J. 314, 713–721
31. Hemesath, T., Steingrimsson, E., McGill, G., Hansen, M. J., Vaught, J., Hodgkinson, C. A., Arnheiter, H., Copeland, N. G., Jenkins, N. A., and Fisher, D. E. (1994) Genes. Dev. 8, 2770–2780
32. Schatt, M. D., Rusconi, S., and Schaffner, W. (1990) EMBO J. 9, 481–487
33. Carter, R. S., and Avadhani, N. G. (1994) J. Biol. Chem. 269, 4381–4387
34. Bungert, J., Koher, I., During, F., and Seifart, K. H. (1992) J. Mol. Biol. 223, 885–898
35. Carbone, J., Lobos, S., Merino, A., Buckhinder, L., Weinmann, R., Natarajan, V., and Reinberg, D. (1989) J. Biol. Chem. 264, 7704–7714
36. Chiang, C.-M., and Roeder, R. G. (1995) Science 267, 531–536
37. Coleman R. A., and Pugh, B. F. (1995) J. Biol. Chem. 270, 13850–13859
38. Roy, A. L., Meisterernst, M., Pognonec, P., and Roeder, R. G. (1991) Nature 354, 245–248
39. Du, H., Roy, A. L., and Roeder, R. G. (1993) EMBO J. 12, 501–511
40. Kollmar, R., Sukow, K. A., Spong, S. K., and Farnham, P. J. (1994) J. Biol. Chem. 269, 2252–2257
41. Lu, J., Lee, W., Jiang, C., and Keller, E. B. (1994) J. Biol. Chem. 269, 5391–5402
42. Faber, P. W., van Rooij, H. C. J., Schipper, H. J., Brinkmann, A. O., and Trapman, J. (1993) J. Biol. Chem. 268, 9296–9301
43. Kirschbaum, B., Pognonec, P., and Roeder, R. G. (1992) Mol. Cell. Biol. 12, 5094–5101