# Differential Expression and Butyrate Response of Human Alkaline Phosphatase Genes Are Mediated by Upstream DNA Elements

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ABSTRACT: Human placentas express high levels of the placental alkaline phosphatase (PLAP) gene and low levels of a highly related gene, germ cell AP (GCAP). Malignant transformation of the placenta is accompanied by a reversal of this pattern of expression. Three Sp1-binding GC-rich DNA elements (sites I−III) located within the first 156 base pairs upstream of the GCAP gene have been shown to direct optimal GCAP gene expression in choriocarcinoma cells. Here we show that the first 100 base pairs upstream of the GCAP gene, which contains sites I and II, constitutes a minimal GCAP promoter. The simultaneous presence of both sites I and II is necessary for GCAP expression and its induction by sodium butyrate. The PLAP promoter directs only a very low level of gene expression in choriocarcinoma cells; the expression does not respond to butyrate. The −100/−1 DNA regions between the GCAP and PLAP promoters differ by only eight base pairs. However, the GC-rich stretches in sites I and II of the GCAP promoter are disrupted in the corresponding PLAP promoter. This disruption blocks or markedly reduces the binding of choriocarcinoma nuclear factors to the PLAP promoter, leading to a reduction in expression and a loss of butyrate response. We further demonstrate that nucleotides −75 to −58 in both AP promoters, which bind a human Y-box binding protein, appear to down-regulate GCAP expression.

There are four human alkaline phosphatase (AP) isozymes: a placental form (PLAP),[1] a germ cell form (GCAP) that is very similar to PLAP, an intestinal form, and a liver/bone/kidney form (Knoll et al., 1987, 1988; Henthorn et al., 1988; Millan & Mains, 1988; Weiss et al., 1988; Watanabe et al., 1989). Each isozyme is encoded by a separate gene. GCAP and PLAP are closely related, sharing 98% amino acid homology, and are encoded by structurally nearly identical genes (Knoll et al., 1987, 1988; Millan & Mains, 1988; Watanabe et al., 1989). Despite the high degree of homology between these two genes, the two enzymes exhibit different patterns of expression. For instance, human term placentas express high levels of PLAP mRNA and only low levels (approximately 2% of PLAP) of GCAP mRNA (Povinelli & Knoll, 1991).

Ectopic expression of PLAP and GCAP has been associated with a variety of human tumors (Chang et al., 1980; Lange et al., 1982), including choriocarcinoma, which is an extraembryonic germ cell tumor of the placenta. In cells derived from choriocarcinomas, the expression profiles of PLAP and GCAP are reversed; these cells express a high level of GCAP and only a low level of PLAP (Watanabe et al., 1989). It therefore appears that malignant transformation of the placenta involves an inactivation of PLAP and an activation of GCAP expression. Studies of the control of AP expression in choriocarcinoma cells may yield insight into the mechanisms of malignant transformation of the placenta. To this end, we characterized DNA elements essential for GCAP expression in choriocarcinoma cells (Wada & Chou, 1993). Using transient expression, DNA mobility shifts, and DNase I footprinting analyses, we demonstrated that nucleotides −156 to −1 upstream of the transcription initiation site of the GCAP gene are necessary for optimal promoter activity (Wada & Chou, 1993). There are three GC-rich activation sites within this region that are necessary for GCAP expression, and all three sites bind to an Sp1-like nuclear factor (Wada & Chou, 1993). Here, we demonstrate that the marked reduction of PLAP expression in choriocarcinoma cells correlates with the absence in the PLAP promoter of two of the GC-rich activator elements found in the GCAP promoter.

GCAP gene transcription in choriocarcinoma cells has been shown to be stimulated by sodium butyrate, resulting in increased mRNA expression and enzyme biosynthesis (Pan et al., 1991). In contrast, expression of the PLAP gene in these cells is unaffected by butyrate (Povinelli et al., 1992). Butyrate inhibits histone deacetylase, resulting in an increase in histone acetylation and generalized changes in chromatin structure (Candido et al., 1978; Sealy & Chalkley, 1978). However, butyrate affects the expression of only a few genes, suggesting other, more specific mechanisms of action. DNA regulatory elements in a number of promoters that appear to confer butyrate inducible transcription have been identified by transfection studies. These include the human immunodeficiency virus-1 long terminal repeat (HIV-LTR) (Bohan et al., 1989), the Moloney murine sarcoma virus (MSV) enhancer (Tang & Taylor, 1990), a chicken embryonic globin gene enhancer (Glauber et al., 1991), and the cytotoxic cell protease-1 (CP-1) promoter (Fregeau et al., 1992). In the

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[1] Abbreviations: PLAP, placental alkaline phosphatase; GCAP, germ cell alkaline phosphatase; HIV-LTR, human immunodeficiency virus-1 long terminal repeat; MSV, Moloney murine sarcoma virus; CP-1, cytotoxic cell protease-1; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; TAF, TATA-binding protein-associated factor.

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HIV-LTR, the MSV enhancer, and the CP-1 promoter, the butyrate-responsive regions map to GC-rich sequences, including Sp1 binding sites. In the present report, we show that GC-rich sequences encompassing Sp1 binding sites in the GCAP promoter are the targets of butyrate action. PLAP, which lacks these sequences, does not have the ability to respond to butyrate. We also demonstrate that a human Y-box transcription factor binds to both the GCAP and PLAP promoters at nucleotides −75 to −58. The Y-box binding motif appears to be involved in negative GCAP gene regulation.

## MATERIALS AND METHODS

*Construction of Promoter−CAT Fusion Genes.* The PLAP promoter−chloramphenicol acetyltransferase (CAT) fusion gene constructs were synthesized by polymerase chain reaction (PCR) using a PLAP genomic clone as a template. The 3′ primer for the PLAP 5′ deletion mutants is nucleotides −18 to −1, and the 5′ primers are nucleotides −569 to −552, −206 to −189, −100 to −83, and −49 to −32, respectively. Each primer contains an additional *Hin*dIII or *Xba*I site at the 5′ end. After digestion with *Xba*I and *Hin*dIII, the amplified fragments were inserted upstream of the bacterial CAT gene of a modified promoterless and enhancerless pCAT-Basic plasmid (pBCAT, Lei et al., 1992). Some of the constructs were inserted in the modified pCAT-enhancer plasmid, which contains the SV40 enhancer. Mutagenesis was carried out as described by Higuchi (1990). All constructs were verified by DNA sequencing. The pSVCAT, which contains both SV40 enhancer and promoter, and pBCAT (Lei et al., 1992) plasmids were used as positive and negative controls, respectively.

*Transfection and CAT Assays.* JEG-3 human choriocarcinoma cells (Watanabe et al., 1989) in 150 $cm^2$ flasks were transfected in suspension by the calcium phosphate−DNA coprecipitation method (Chu & Sharp, 1981; Luthman & Magnusson, 1983) as previously described (Wada & Chou, 1993). The CAT activity was assayed by incubating total cellular protein in a buffer containing 250 mM Tris (pH 7.8), 4 mM acetylcoenzyme A, and 0.1 $\mu$Ci [$^{14}$C]chloramphenicol (Fordis & Howard, 1986). Routinely, the assay was run for 1 h with the amount of extract required to convert 0.5−50% of the substrate to the acetylated forms. Assays outside this range were repeated using the appropriate amount of extract. The acetylated compounds were separated from chloramphenicol by thin-layer chromatography (95% chloroform−5% methanol, v/v) on silica gel IB2 (Gilman Sciences). Spots were quantitated on an AMBIS Radioanalytic Imaging System (San Diego, CA).

*Library Screening and Characterization of cDNA Clones.* A human placental $\lambda$gt11 cDNA library (obtained from Dr. F. Gonzales, NIH) was screened with a double-stranded oligonucleotide probe comprising a concatenated trimer of nucleotides −76 to −58 (5′-GGTCAAGGTGGTAACAAGG-3′) in the GCAP promoter following the procedures of Ausubel et al. (1992). Approximately $10^6$ plaques were screened, and two positive clones were obtained. The cDNA inserts from positive clones were ligated into pGEM vectors (Promega Biotechnology, Madison, WI) and characterized by DNA sequencing.

*Gel Mobility Shift Assay.* End-labeled oligonucleotide probes (1 ng; 0.5−1 × $10^5$ cpm) were incubated for 20 min at room temperature in binding reaction buffer [10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.05% NP-40, 1 mM EDTA, 0.5 mM DTT, and 10% glycerol] containing 1 $\mu$g of poly(dI-dC) and 3 $\mu$g of nuclear extracts prepared as previously described (Wada & Chou, 1993). Following binding, the mixture was electrophoresed through a 5% nondenaturing polyacrylamide gel and autoradiographed. For competition experiments, competitor DNA was incubated in the mixture prior to the addition of nuclear extract. The anti-FRGY1 antiserum was kindly provided by Dr. A. P. Wolffe at the NICHD, and the anti-Sp1 monoclonal antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

## RESULTS

*Butyrate Induces GCAP Expression and Is Mediated via Activation Elements in the GCAP Promoter.* We have previously shown that sodium butyrate increases the rate of GCAP gene transcription, resulting in an increase in GCAP mRNA expression, enzyme synthesis, and total phosphatase activity (Watanabe et al., 1989; Pan et al., 1991). To examine whether the butyrate action is mediated via regulatory elements in the GCAP promoter, we measured CAT activity directed by a series of GCAP promoter−CAT fusion constructs transiently transfected into JEG-3 human choriocarcinoma cells in the absence and presence of sodium butyrate (Figure 1A). GCAP promoter−CAT constructs that contain greater than 100 base pairs of upstream sequence were capable of directing CAT expression. Moreover, CAT activities directed by these GCAP constructs were stimulated 7.6−9.7-fold by sodium butyrate (Figure 1A). Deleting GCAP promoter sequences upstream of −49 abolished CAT expression as well as the stimulatory effect of butyrate. In earlier studies (Wada & Chou, 1993), we have shown that the GCAP promoter contains three GC-rich activation elements, I (−63/−44), II (−87/−67), and III (−136/−103) (Figure 1B), which are necessary for optimal GCAP promoter activity in JEG-3 cells. The correlation between the increase in CAT expression by butyrate and the presence of activation elements I and II suggested that butyrate action is mediated via site I and/or II in the GCAP promoter.

To address this, we disrupted the GC-rich stretches in activator sites I and II of the GCAP(−100/−1)CAT construct by changing the sequences to those of the related, but inactive, PLAP promoter (see Figure 1B and below) and analyzed CAT activity in the absence and presence of butyrate (Table 1). Disrupting site I [GCAP(−100/−1)-CATSIAA, GG at positions −53 and −52 to AA] or site II [GCAP(−100/−1)CATSIIA, G at nucleotide −78 to A] diminished CAT activity to approximately 10% of that of GCAP(−100/−1)CAT. Disrupting both sites I and II [GCAP(−100/−1)CATSIAASIIA] abolished CAT expression (Table 1). While CAT expression directed by GCAP-(−100/−1)CAT was stimulated 9.3-fold by butyrate, only a moderate 1.4- and 3.7-fold increase in CAT expression was observed with GCAP(−100/−1)CATSIAA and GCAP(−100/−1)CATSIIA, respectively (Table 1). The double mutant GCAP(−100/−1)CATSIAASIIA lost both the ability to direct CAT expression and the ability to be stimulated by butyrate. These results indicated that butyrate action was mediated through activation elements I and II in the GCAP promoter and that the simultaneous presence of both of these sites was necessary for maximal induction by butyrate.

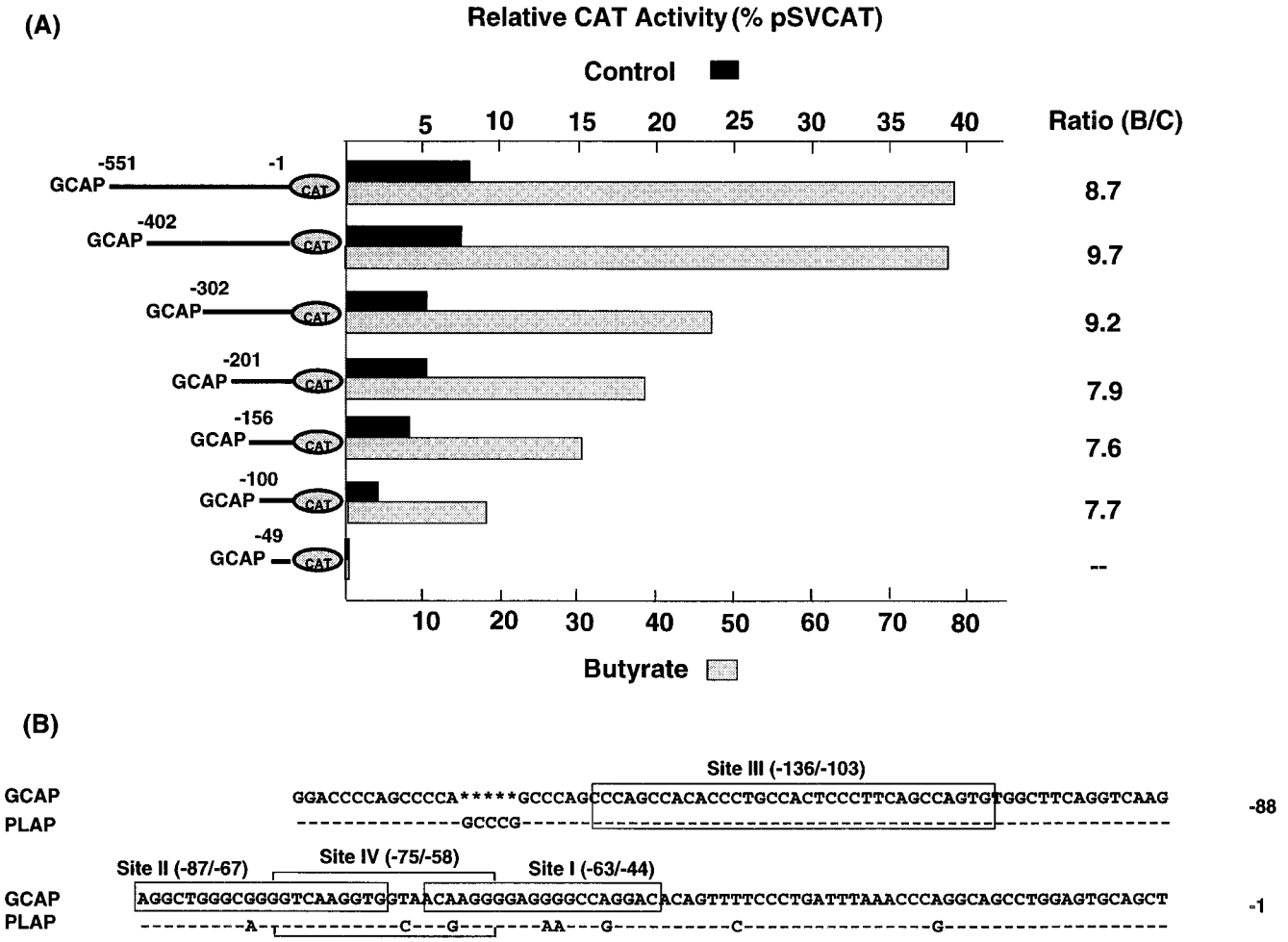


FIGURE 1: (A) Induction of GCAP gene expression by sodium butyrate. Nucleotides −551/−1, −402/−1, −302/−1, -201/−1, −156/−1, −100/−1, or −49/−1 from region 5′ of the transcription initiation site of the GCAP gene were inserted upstream of a modified promoterless and enhancerless CAT gene, pBCAT (Lei et al., 1992). The pSVCAT plasmid which contains both SV40 enhancer and promoter and the pBCAT plasmid were used as positive and negative controls, respectively. The CAT activity was determined for each construct after transient transfection into JEG-3 choriocarcinoma cells for 3 days in the presence (B) or absence (C) of 2 mM sodium butyrate. Data are the mean of three independent experiments using two batches of plasmids. (B) Sequences of nucleotides −156/−1 in the GCAP and PLAP genes. The three GC-rich Sp1 binding motifs essential for GCAP expression in choriocarcinoma cells (Wada & Chou, 1993) are bracketed. A Y-box binding protein has been shown in this study to bind to site IV of both promoters.

Table 1: Functional Analysis of GCAP and PLAP 5′-Flanking Regions[a]

| promoter−CAT fusion gene | relative CAT activity (%) control | relative CAT activity (%) butyrate | B/C |
|---|---|---|---|
| GCAP(−100/−1)CAT | 100 | 934 ± 17.5 | 9.3 |
| GCAP(−100/−1)CATSIAA | 8.4 ± 1.6 | 11.7 ± 2.3 | 1.4 |
| GCAP(−100/−1)CATSIIA | 11.6 ± 2.4 | 43.0 ± 7.5 | 3.7 |
| GCAP(−100/−1)CATSIAASIIA | ND | ND | |
| GCAP(−75/−1)CAT | 4.5 ± 0.5 | 8.7 ± 0.4 | 1.9 |
| GCAP(−66/−1)CAT | 11.7 ± 0.8 | 22.2 ± 1.7 | 1.9 |
| PLAP(−100/−1)CAT | ND | ND | |
| PLAP(−100/−1)CATSIGGSIIG | 93.5 ± 13 | 1107 ± 172 | 11.8 |

[a] AP promoter−CAT constructs were transfected into JEG-3 choriocarcinoma cells, and pSVCAT and pBCAT were used as positive and negative controls, respectively. CAT activity is expressed as a ratio of the activity of GCAP(−100/−1)CAT. Data are the mean ±SE of three independent experiments. ND is nondetectable.

We have previously shown that choriocarcinoma nuclear factors, including the transcription factor Sp1, bind to sites I and II of the GCAP promoter (Wada & Chou, 1993). To characterize the effects of butyrate on choriocarcinoma factors binding to the GCAP promoter, we performed gel mobility shift assays using JEG-3 nuclear extracts prepared from cells grown in the absence or presence of sodium butyrate. Two double-stranded oligonucleotide probes, GCAP(−67/−38) encompassing site I (−63/−44) (Figure 2A) and GCAP(−96/−66) encompassing site II (−87/−67) (Figure 2B), were employed. As shown in our earlier studies (Wada & Chou, 1993), two protein−DNA complexes (CI and CII) were formed with either GCAP(−67/−38) (Figure 2A, lane 2) or GCAP(−96/−66) (Figure 2B, lane 2) probe and the formation of both complexes could be effectively competed for by homologous target DNA (Figure 2A, lane 3, and Figure 2B, lane 3). Butyrate did not increase the amounts or the affinity of choriocarcinoma factors specific

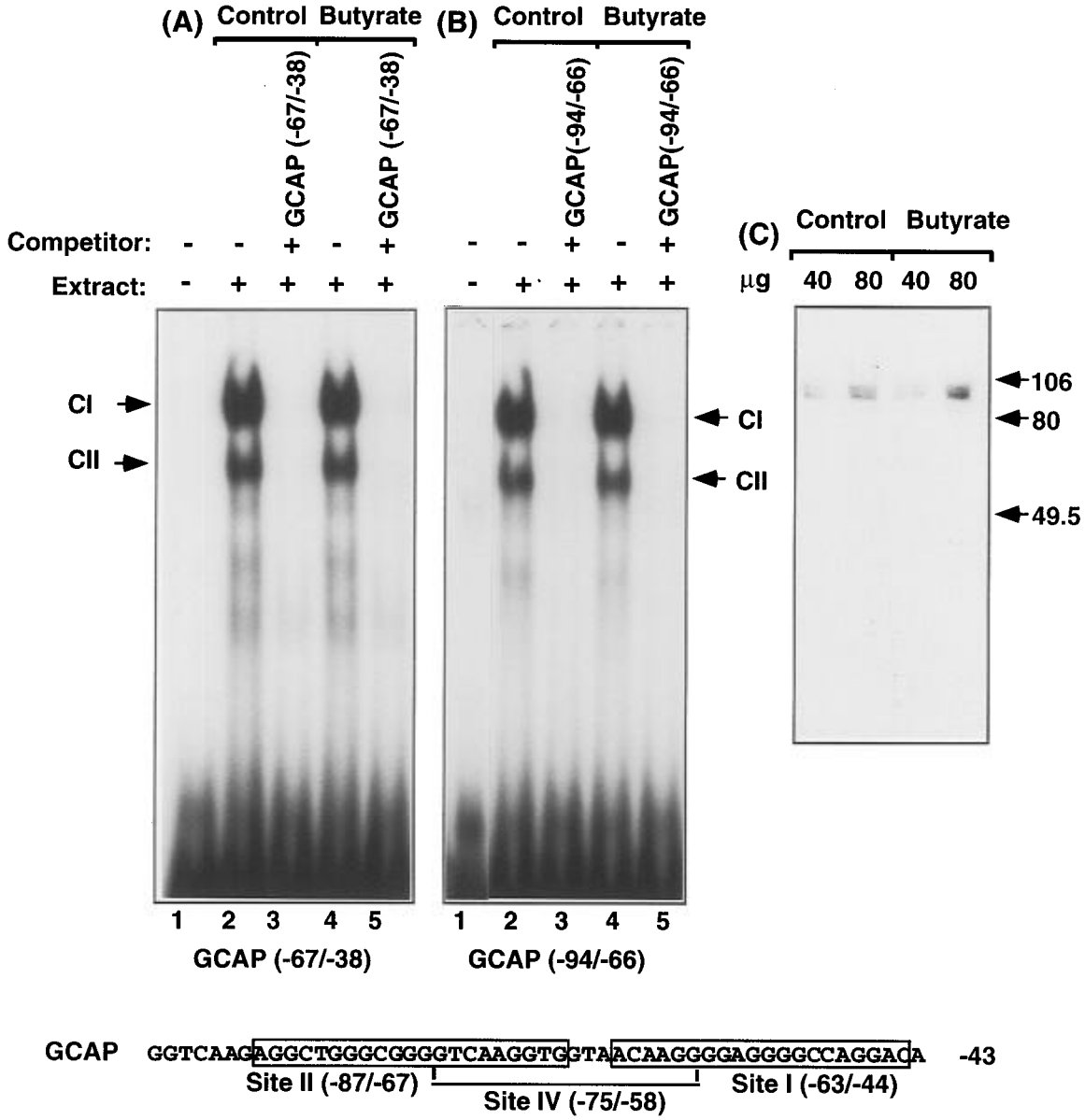


FIGURE 2: Effects of butyrate on binding of JEG-3 nuclear proteins to activator sites in the GCAP promoter. Gel mobility shift assays using end-labeled GCAP(−67/−38) (A) or GCAP(−94/−66) (B) oligo and nuclear extracts prepared from JEC-3 cells grown in the absence or presence of 2 mM sodium butyrate for 3 days. Specific complexes are indicated with arrows. The amount of competitor used is a 100-fold molar excess over the corresponding probe. (C) Western blot analysis of control or butyrate-treated choriocarcinoma nuclear extracts using a monoclonal anti-Sp1 antibody.

to site I or II in the GCAP promoter (Figure 2A, lane 4, and Figure 2B, lane 4). Western blot analysis was then performed to examine if butyrate treatment alters the amount of Sp1 in JEG-3 cells. As shown in Figure 2C, similar amounts of immunoreactive Sp1 were found in nuclear extracts of control and butyrate-treated JEG-3 cells.

*Differential AP Expression in Choriocarcinoma Cells is Controlled by Activation Sites I and II.* In human choriocarcinoma cells, PLAP is expressed at very low levels compared to GCAP (Watanabe et al., 1989) and the PLAP promoter is considerably less active than the GCAP promoter (Povinelli & Knoll, 1991). To determine whether the low level of PLAP expression in JEG-3 cells was due to differences between activation sites I and II in the GCAP and PLAP promoters (site III is identical in both promoters, Figure 1B), we examined expression of PLAP promoter−CAT fusion genes in JEG-3 cells (Table 2). Only the longest PLAP promoter construct [PLAP(−569/−1)CAT] directed low levels of CAT expression in JEG-3 cells, and that was only 6% of the CAT activity expressed by the corresponding GCAP(−551/−1)CAT construct. In these cells, PLAP(−206/−1)CAT, PLAP(−100/−1)CAT, and PLAP(−49/−1)-CAT directed no detectable CAT activity, although promoter

Table 2: Promoter Activity of PLAP 5′-Flanking Regions[a]

| promoter−CAT fusion gene | relative CAT activity (%) |
|---|---|
| GCAP(−551/−1)CAT | 100 |
| PLAP(−569/−1)CAT | 6 ± 2 |
| PLAP(−206/−1)CAT | ND |
| PLAP(−100/−1)CAT | ND |
| PLAP(−49/−1)CAT | ND |
| PLAP(−49/−1)CAT−enhancer | 270 ± 34 |

[a] AP promoter−CAT constructs were transfected into JEG-3 choriocarcinoma cells, and pSVCAT and pBCAT were used as positive and negative controls, respectively. CAT activity is expressed as a ratio of the activity of GCAP(−551/−1)CAT. Data are the mean ±SE of three independent experiments. ND is nondetectable.

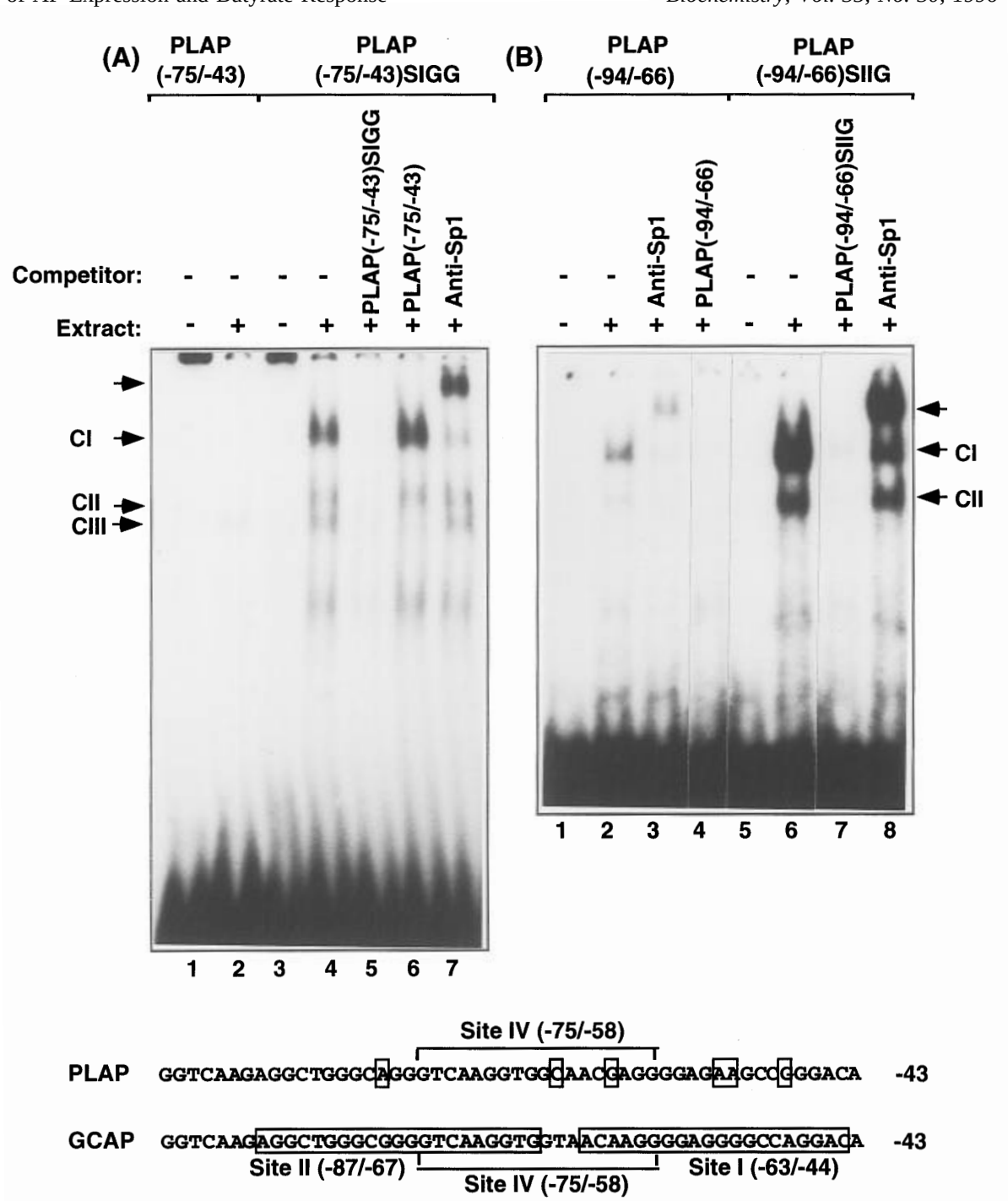


FIGURE 3: Binding of JEG-3 nuclear proteins to putative activator sites in the PLAP promoter. PLAP(−75/−43) (A), PLAP(−75/−43)GG (A), PLAP(−94/−66) (B), and PLAP(−94/−66)G (B) oligos were end-labeled and used in gel mobility shift assays with JEG-3 nuclear extracts. Specific complexes are indicated with arrows. The amount of competitor used is a 100-fold molar excess over the corresponding probe, and the anti-Sp1 antibody used was 1 $\mu$g per reaction.

elements are clearly present in the PLAP promoter. This was demonstrated by the presence of high CAT activity when PLAP(−49/−1) was fused to an SV40 enhancer [PLAP(−49/−1)CAT-enhancer] (Table 2). None of the PLAP fusion gene constructs responded to sodium butyrate. Changing sites I and II to the sequence present in the GCAP promoter [PLAP(−100/−1)CATSIGGSIIG] restored promoter activity and butyrate inducibility to that of the GCAP(−100/−1)-CAT construct (Table 1).

These results suggested that choriocarcinoma factors that bind to the GCAP promoter either do not bind to or bind with lower affinities to the PLAP promoter. We therefore performed gel mobility shift assays using JEG-3 nuclear extracts and three double-stranded oligonucleotide probes: PLAP(−67/−38) encompassing site I at nucleotides −63/−44, PLAP(−75/−43) encompassing site I at nucleotides −63/−44 and site IV at −75/58, and PLAP(−94/−66) encompassing site II at −87/−67. No detectable protein−DNA complex was observed with PLAP(−67/−38) (data not shown), but a weak complex (CIII, Figure 3A, lane 2) was observed with the PLAP(−75/−43) probe. Three protein−DNA complexes were formed (CI, CII, and CIII, Figure 3A, lane 4) when bases −52 and −53 were changed from AA to GG [PLAP(−75/−43)SIGG], restoring the GCAP sequence. The formation of all three complexes was effectively competed for by PLAP(−75/−43)SIGG (Figure 3A, lane 5),

whereas complex III formation was selectively abolished by the parental PLAP(−75/−43) oligo (Figure 3A, lane 6), suggesting that complex III involves a protein factor binding to site IV (Figure 1B).

Site II differs by one base at position −78 between PLAP (A at −78) and GCAP (G at −78). Two complexes (CI and CII, Figure 3B, lane 2) with markedly reduced affinities were formed when PLAP(−94/−66) was the target DNA. As expected, protein−DNA complexes of greatly increased affinities were formed when using PLAP(−94/−66)SIIG as the target DNA (Figure 3B, lane 6) where base −78 in PLAP was changed from an A to the G found in the GCAP promoter.

Earlier supershift studies have shown that one of the factors that binds to both sites I and II (Figure 3B, lane 8) in the GCAP promoter is Sp1 (Wada & Chou, 1993). Antibody to Sp1 also interacted with factors bound to site II in the PLAP promoter (CI, Figure 3B, lane 3). However, antibody to Sp1 did not react with complex III formed with the PLAP site I probe (Figure 3A, lane 7). Among the three complexes formed with the PLAP(−75/−43)SIGG mutant probe, only complex I was supershifted by anti-Sp1 antibody (Figure 3A, lane 7). Thus, in contrast to the GCAP site I, Sp1 does not bind to site I in the PLAP promoter.

*Y-Box Binding Factors Play a Role in AP Expression.* To examine further the role of site IV binding factor on AP expression, we determined CAT activity directed by two GCAP constructs, one containing sites I and IV [GCAP(−75/−1)CAT] and the other containing only site I [GCAP-(−66/−1)CAT]. Both constructs had reduced levels of CAT activity in the presence or absence of butyrate (Table 1). However, the GCAP(−66/−1)CAT construct directed a reproducibly higher CAT activity than the GCAP(−75/−1)CAT plasmid, suggesting that nucleotides −75 to −66 in the GCAP promoter encompassing part of site IV may have an inhibitory action.

Three complexes (CI, CII, and CIII) were formed in gel mobility shift assays using a GCAP(−75/−43) probe containing sites I and IV (Figure 4A, lane 2). Complex III formation was specifically competed for by both GCAP(−75/−58) and PLAP(−75/−58) site IV oligos (Figure 4A, lanes 4 and 5), suggesting that the same factor binds to this region of both promoters. Indeed, when GCAP(−75/−58) and PLAP(−75/−58) site IV probes were used as the target DNA, only complex III was formed (Figure 4B, lane 2, and Figure 4C, lane 2).

To determine the nature of the protein factor that binds to site IV, we screened a human placental cDNA expression library using a probe with three copies of an oligonucleotide corresponding to nucleotides −76 to −58 of the GCAP promoter. Two strongly hybridizing clones were identified from a screen of $10^6$ plaques. Sequence analysis revealed that the two clones overlapped and coded for a human Y-box binding protein. Studies have shown that the N-terminal cold shock DNA binding domains of Y-box transcription factors are highly conserved among humans, rats, and frogs (Tafuri & Wolffe, 1991). An antiserum to a *Xenopus* Y-box transcription factor (FRGY1) inhibited the formation of complex CIII with either GCAP(−75/−58) (Figure 4B, lane 5) or PLAP(−75/−58) (Figure 4C, lane 5). This suggests that this antiserum is reacting with the DNA binding domain of a Y-box binding protein that binds to site IV of the GCAP promoter, resulting in the down-regulation of GCAP expression in choriocarcinoma cells.

## DISCUSSION

Placental malignancy is accompanied by an activation of GCAP expression and an inactivation of PLAP expression. In order to gain insight into the molecular mechanisms responsible for this switch, we have characterized the GCAP and PLAP promoters in JEG-3 cells derived from malignant trophoblasts. In earlier studies (Wada & Chou, 1993), we have shown that the GCAP promoter contains three GC-rich activation sites, I (63/−44), II (−87/−67), and III (−136/−103), which are essential for optimal GCAP expression in these cells. Activation site III in GCAP and PLAP is identical, whereas the GC-rich stretches within sites I and II of GCAP were disrupted in the corresponding region of the PLAP promoter (GG to AA at −53/−52 in site I and G to A at −78 in site II). This disruption greatly reduces PLAP expression in choriocarcinoma cells. Restoring the GC stretches in the PLAP promoter [PLAP(−100/−1)CATSI-GGSIIG] increases CAT activity to that observed with the GCAP(−100/−1)CAT construct. Thus, the differential AP expression observed in choriocarcinoma cells is due to three base pair differences between activation sites I and II in the two promoters.

Sodium butyrate greatly stimulates the expression of GCAP but not PLAP in JEG-3 cells. We present evidence that induction of GCAP expression by butyrate is mediated by activator sites I and II. First, CAT expression directed by GCAP(−100/−1)CAT, which contains both sites I and II, was stimulated 9.3-fold by butyrate. Mutating either site I [GCAP(−100/−1)CATSIAA] or site II [GCAP(−100/−1)CATSIIA] reduced the butyrate response. Mutating both sites [GCAP(−100/−1)CATSIGGSIIA] abolished butyrate induction. Second, PLAP promoter−CAT constructs failed to respond to butyrate unless sites I and II in PLAP were restored to the same GC-rich [PLAP(−100/−1)CATSIGGSI-IG]CAT sequence as that of GCAP. In the presence of butyrate, CAT activity directed by [PLAP(−100/−1)CATSIGGSIIG]CAT was as high as those observed with the GCAP(−100/−1)CAT construct. We demonstrated that the increase in GCAP transcription in response to butyrate is not due to an increase in the amount or affinity of choriocarcinoma factors, including Sp1, to the GCAP promoter, suggesting other modes of action. Sodium butyrate has been shown to inhibit histone deacetylase, resulting in the accumulation of hyperacetylated histones and an altered chromatin structure (Candido et al., 1978; Sealy & Chalkley, 1978). However, not all gene expression is affected by butyrate. Indeed, DNA regulatory sequences have been identified which mediate the butyrate response in a number of genes (Bohan et al., 1989; Tang & Taylor, 1990; Glauber et al., 1991; Fregeau et al., 1992). In the HIV-LTR (Bohan et al., 1989), the MSV enhancer (Tang & Taylor, 1990), and the CP-1 promoter (Fregeau et al., 1992), the response has been mapped to GC-rich Sp1-binding sequences similar to those found in the GCAP promoter. This suggests that modulation of the expression of GCAP and the aforementioned genes by butyrate may be mediated by similar molecular mechanisms. Studies have shown that the Sp1 protein is modified post-translationally by protein glycosylation (Jackson & Tjian, 1988) and becomes phosphorylated upon binding to promoter elements (Jackson et al., 1990).

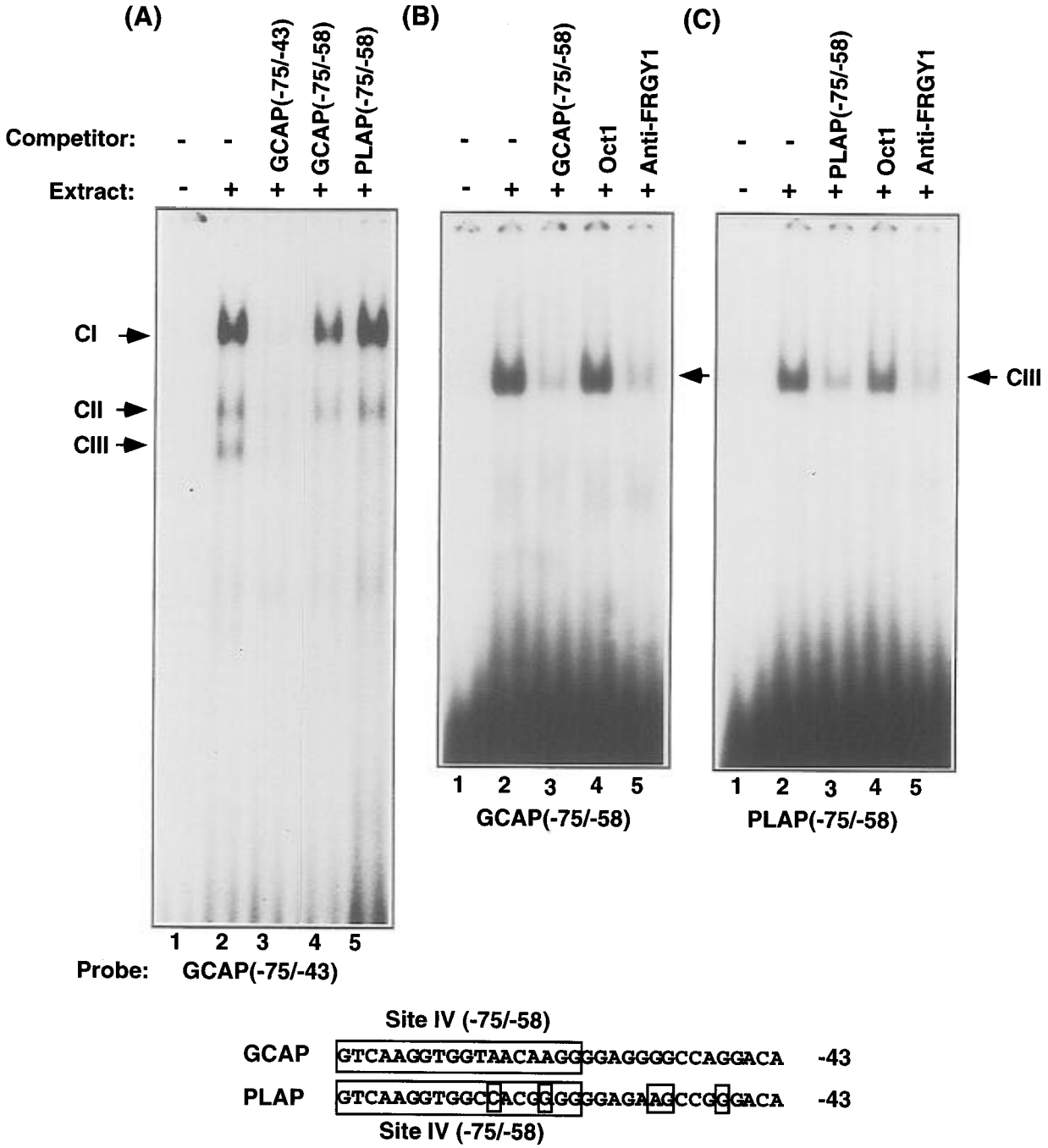


FIGURE 4: Binding of JEG-3 nuclear proteins to putative sites in the GCAP and PLAP promoters. GCAP(−75/−43) (A), GCAP(−75/−58) (B), and PLAP(−75/−58) (C) oligos were end-labeled and used in gel mobility shift assays with JEG-3 nuclear extracts. Specific complexes are indicated with arrows. The amount of competitor used is a 100-fold molar excess over the corresponding probe, and the anti-FRGY1 antibody used was 2 $\mu$g per reaction.

Both affect the ability of Sp1 to activate transcription, and butyrate may modulate either or both processes without affecting the absolute amounts of Sp1. Moreover, transcriptional activation by Sp1 requires TATA-binding protein-associated factors (TAFs) which act as coactivators (Pugh & Tjian, 1990). TAF110, an Sp1 coactivator, has been shown to interact with the glutamine-rich activation domains of Sp1 and mediate transactivation (Hoey et al., 1993). Induction of GCAP expression by butyrate may be mediated through butyrate-sensitive TAFs essential for Sp1 activation.

Earlier studies have demonstrated that GCAP promoter sites I and II bind to choriocarcinoma nuclear factors including Sp1. PLAP site II also binds these factors, albeit at greatly reduced affinity. However, PLAP site I has no detectable affinity for these choriocarcinoma factors. The preferential expression of the GCAP but not the PLAP gene in choriocarcinoma cells demonstrates that binding of choriocarcinoma factors including Sp1 to sites I and II is essential for optimal AP expression.

We have also shown that the protein factor that binds to site IV (−75 to −58) in both GCAP and PLAP promoters is a Y-box binding protein. Screening a placental cDNA expression library with a site IV probe [GCAP(−75/−58)] yielded two overlapping clones that encode the human Y-box binding protein (Zeleznik-Le et al., 1991). Furthermore, the formation of complex (CIII) between choriocarcinoma nuclear extracts and GCAP(−75/−58) or PLAP(−75/−58) oligo was inhibited specifically by an antiserum raised against

a *Xenopus* Y-box homolog, FRGY1 (Tafuri & Wolffe, 1991). Site IV in GCAP and PLAP differs by two nucleotides, confirming the relative sequence divergence in the Y-box binding protein motifs (Hasegawa et al., 1991). Interestingly, site IV, encompassing the Y-box binding motif, appears to down-regulate GCAP expression in choriocarcinoma cells. It is possible that GCAP expression is repressed in cells that express high levels of the Y-box protein.

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