Transcriptional Activation of Fibroblast Growth Factor 1.B Promoter Is Mediated through an 18-Base Pair cis-Acting Element* (Received for publication, October 22, 1996, and in revised form, January 10, 1997)

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Four different transcripts encoding fibroblast growth factor 1 (FGF-1, also known as aFGF) have been previously identified in our laboratory. Among them, FGF-1.B is the major transcript expressed specifically in the neuronal cells in brain tissue. Using the transient transfection experiment in a glioblastoma cell line, U1240MG, that expresses 1.B, we previously identified two regulatory regions (RR1 and RR2) in the brain-specific promoter, FGF-1.B. In the present study, we showed that the minimal region required for the DNA-protein interaction in RR2 resides in an 18-base pair (−484 to −467) sequence, by using DNase I footprinting and methylation interference studies and electrophoretic mobility shift assays. This minimal cis-acting element was found to be sufficient in enhancing the reporter activity driven by the heterologous herpes simplex virus thymidine kinase promoter in a cell line-specific manner. UV cross-linking analysis of this 1.B-specific complex revealed a 18-bp sequence (−467) which can form more stable complexes than the −484 to −467 sequence. This enhancing effect, however, was not detected in a glioblastoma cell line, U1242MG, which is negative for 1.B expression. By electrophoretic mobility shift assays, we also identified a specific DNA-protein complex, namely complex I, which is specific for 1.B-positive cell lines and human brain tissue. By in situ UV cross-linking experiment, we further showed that complex I contains two major DNA-binding proteins of apparent molecular masses of 37 and 98 kDa. Our results suggest that the formation of complex I, resulting from the heterodimerization of a 37-kDa protein (1.B-specific) and a 98-kDa protein (ubiquitous) may likely be a prerequisite for the enhanced expression of 1.B transcript in neuronal cells.

The FGF family consists of 14 structurally related polypeptide growth factors (1, 2). FGF-1, a prototype member, was originally identified as a mitogen for endothelial cells (3) and subsequently for a variety of mesoderm- and neuroectoderm-derived cells (4, 5). This growth factor is involved in a variety of physiological and pathological function such as tissue growth, wound healing, neovascularization, mesoderm development, and angiogenesis (6–10). FGF-1 confers tumorigenicity when introduced via expression vectors into normal cells (11–13). Unlike FGF-2, FGF-1 is found primarily in brain and retina, although low levels of its mRNA have been demonstrated in other tissues (14–16). FGF-1 may function in establishment of retinal fate and ganglion cell differentiation from the invaginated optic vesicle (17). However, the role of FGF-1 in brain is not yet well understood. By in situ hybridization and immunohistochemical analysis, it has been shown that the expression of this growth factor in brain is exclusively in neural cells but not in glial cells (18, 19). It was also shown that the expression of FGF-1 mRNA in neurons is correlated with specific developmental events (20). FGF-1 was reported to coexist with tyrosine hydroxylase, a key enzyme for catecholamine synthesis, in neuronal cells (21) and is involved in the expression of this gene (22). FGF-1 mRNA (23) and the protein (24) levels were also reported to elevate in lesioned rat brain. Interestingly, in gnomas, a major human intracranial tumor (25), FGF-1 mRNA expression was reported to increase significantly (7, 26). However, the regulatory mechanism of FGF-1 gene expression is only beginning to be understood.

The human FGF-1 gene spans over 120 kilobase pairs containing three protein coding exons and at least four upstream untranslated exons, namely 1A, 1B, 1C, and 1D (27–31). Splicing of each of these untranslated exons to the first protein coding exon generates four different mRNA transcripts designated as 1.A, 1.B, 1.C, and 1.D. These multiple transcripts generated by alternate promoter usage and splicing are distributed in a tissue-specific manner. The major transcript in human brain, 1.B, is different from that in human kidney or prostate (31, 32). By RNase protection assay, it was shown that 1.B transcripts are highly expressed in glioblastoma tissues, as well as in some of the glioblastoma cell lines (26, 32). By in situ hybridization with cRNA probe specific for the murine FGF-1.B transcript, our laboratory has recently shown that 1.B mRNA is expressed in mouse brain and restricted largely to sensory and motor nuclei in the brainstem, and to the ventral spinal cord and cerebellum (33).

We have previously identified a 41-bp DNA sequence (−507 to −467) in the brain-specific FGF-1.B promoter, designated as regulatory region 2 (RR2), through luciferase reporter assays (34). RR2 binds nuclear factors through a 23-bp (−489 to −467) region and this binding is linked to the enhanced functional activity of the promoter (34). In the present communication, we have further characterized the functional importance of this region. We have narrowed down the minimal cis-acting sequence to an 18-bp region (−484 to −467) which can form more than one DNA-protein complex including the 1.B-specific complex, complex I. This 18-bp sequence is sufficient to enhance the luciferase reporter gene expression driven by a heterologous thymidine kinase (tk) promoter in a cell line-specific manner. UV cross-linking analysis of this 1.B-specific complex re-
that it contains a deletion of nucleotides at 492 to 467 of FGF-1.B promoter and the mutants are described in Table I. To localize the minimal region of DNA-protein interaction by electrophoretic mobility shift assays (EMSA), we also synthesized the following oligonucleotides, which include the annealing sequences 484 to 467, 484 to 473, and 480 to 467. The sequences of the pairs of oligonucleotides are, respectively, as follows 5'-GATTCTCGTCCCCCTTGGCAACCTCG and 5'-GATCCGATCTGACGAGTTGCCAGGAGGACAGA, 5'-GATTCTCGTCCCCCTTGGCAACCTCG and 5'-GATCCGATCTGACGAGTTGCCAGGAGGAA, and 5'-GATCCGATCTGACGAGTTGCCAGAGGACACA.

EMSA—The binding reaction contained the binding buffer (55 mM Hepes, pH 7.9, 1 mM MgCl2, 0.5 mM EDTA, 0.5% glyceraldehyde, 5% glycerol) with radiolabeled DNA probe (30,000 cpm), 2 μg of polyd-(dC), 5 μg of nuclear protein, and cold competitor when desired in a total volume of 20 μl. Each reaction was initiated by addition of protein and incubated for 30 min at room temperature, and then 12 μl was analyzed in a 4% polyacrylamide gel in 0.25× TBE buffer (22.25 mM Tris borate, 0.5 mM EDTA, pH 8.0). Following electrophoresis, the gel was dried under vacuum and autoradiographed.

Sodium Deoxycholate (DOC) Treatment of U1240MG NE—To exclude the possibility that formation of additional complexes was due to the interactions occurring between the DNA-binding protein and non-DNA-binding proteins, the NE was treated with DOC as described by Chellapan et al. (37). U1240MG NE (50 μg) was incubated in ice in a total volume of 10 μl with 0.7% DOC (final concentration) for 20 min, and then Nonidet P-40 was added to a final concentration of 1%. One μl of this reaction mix was assayed in EMSA in a total volume of 20 μl. An equivalent amount of untreated NE was also tested for comparison of the binding activity in the presence of 0.05% Nonidet P-40.

DNase I Footprinting Assay—An aliquot containing 0.3 ng (30,000 cpm) of 5'-484 to 357 or 594 to 357 Smal-BbsI fragment of FGF-1.B promoter, labeled at the 3'-end at the BbsI site (sense strand), was incubated with increasing amount of U1240MG nuclear extract (12-60 μg of protein) or 20 μg of acetylated BSA in a total volume of 30 μl containing 25 mM Hepes (pH 7.9), 1 mM MgCl2, 0.5 mM DTT, 40–60 mM KCl, 5% glycerol, and 4 μg of polyd-(dC) (Pharmacia). After 30 min of incubation, an equal volume (30 μl) of a solution containing 8 mM MgCl2 and 4 mM CaCl2 was added, followed by the addition of 10–25 ng of denatured and unlabeled nuclear extract-extracted DNase I (Boehringer Mannheim). The digestions were carried out for 60 s. The reaction was stopped by adding 140 μl of stop buffer (TBE plus 0.1 mM NaCl, 0.2% SDS), 200 μl of phenol, and 200 μl of CHCl3. The aqueous layer was extracted further with 200 μl CHCl3, and ethanol-precipitated in the presence of 5 μg of tRNA. The precipitates were denatured, and equal counts were analyzed in a 6% acrylamide sequencing gel.

Methylation Interference Assay—The HindIII-EcoRI fragment from plasmid pFGF-1.B 492 to 467 was 3'-end-labeled at EcoRI site (sense strand), partially methylated with dimethyl sulfate, and used as a probe in binding reactions containing U1240MG NE. DNA-protein complexes were separated from the free DNA by EMSA, eluted from the gel and cleaved with piperidine, and then analyzed in an 8% sequencing gel as described (34).

UV Cross-linking Experiment—Analysis of the EMSA complexes was done by in situ UV cross-linking according to the method of Wu et al. (38) with a slight modification. The 32P-labeled photoaffinity probe used in this experiment was made by annealing two complementary strands 5'-GATCCGATCTGACGAGTTGCCAGAGGACACA (sense strand) and 5'-GATCCGATCTGACGAGTTGCCAGAGGAA (antisense strand) followed by end-filling with dGTP, 5-bromo-dUTP, [α-32P]dATP, and [α-32P]dCTP by Klenow reaction. The gel shift binding reaction, carried out using the labeled photoaffinity probe (replacing 484 to 467), was scaled up in the absence or presence of a specific cold competitor. The reaction mixture was run in 1% low melting agarose gel in 0.5 × TAE buffer (20 mM Tris acetate, 1 mM EDTA, pH 8.0) at 4°C. The gel was covered with plastic wrap and exposed to UV.
To determine the minimal sequence in RR2 required for the specific DNA-protein interaction, we performed (i) DNase I footprinting assay, (ii) methylation interference assay, and (iii) EMSA with overlapping sequences between −492 and −467.

**DNase I Footprinting Assay**—To characterize the minimal cis-acting element for the specific DNA-protein interaction, we used end-labeled (sense strand labeled at its 3′-end) DNA from −540 to −357 (Fig. 2A) or −594 to −357 (Fig. 2B) in DNase I footprinting assay with increasing amount of U1240MG NE. The results showed a single footprint in the same position (−485 to −465) when either probe was used and indicated that the minimal sequence required for the DNA-protein interaction resides in the sequence from −485 to −467.

**Methylation of Guanine Nucleotide at −482, −474, and −473 in the Sense Strand Strongly Affects the Specific DNA-Protein Complex Formation**—To identify the precise location of the nucleotides in the sense strand contacting with the nuclear factors, methylation interference analysis using a probe labeled at the 3′-end of the sense strand was performed. The previous study could not detect the contact sites in the sense strand (34); this may be due to the full methylation of the genomic fragment (−507 to −467) having only 10 guanine nucleotides in the same strand. This problem was overcome by using the DNA fragment (−492 to −467) flanked by vector sequences at both ends. It is evident from the results (Fig. 3) that the methylation of guanine nucleotides in the sense strand at positions −482, −474, and −473 strongly affects the specific DNA-protein interaction.

The Minimal cis-Acting Element Required for the Specific
protein interaction with the minimal 18-bp (−484 to −467) region and the cell line specificity of such interaction, we tested the activity of the minimal tk promoter in absence or presence of the 18-bp or the 26-bp (−492 to −467) DNA sequence of FGF-1.B in U1240MG or U1242MG cell lines. The results showed that the insertion of the 18-bp sequence upstream of the tk promoter enhanced the reporter activity by 6-fold relative to tk (Fig. 5A, left panel). It is also clear that the 18-bp DNA sequence is sufficient to enhance the activity of the heterologous tk promoter, since the 26-bp construct has no further effect on the enhancement of the promoter activity. This enhancement is cell-specific, since neither 18-bp nor 26-bp DNA sequence enhanced the tk promoter activity in U1242MG cells (Fig. 5A, right panel). It is important to note that a comparable level of reporter activity (10-fold) was scored by both U1240MG and U1242MG cells when tk (−200) was used in transient transfection assay (Fig. 5A). We also assayed the reporter activity of tk promoter in the absence or presence of the 18-bp oligonucleotide in either orientation (Fig. 5B). The results showed that the 18-bp sequence, when placed upstream of the tk promoter in the sense orientation, activated it by 6.5-fold. When placed in the antisense orientation, the activity was reduced to 1.7-fold relative to tk. This result is consistent with the data shown in Fig. 1, demonstrating that the regulatory effect of the 18-bp sequence is orientation-dependent. The insertion of mutant oligonucleotide, mut 456 and mut 56, upstream of the tk (mut 456/tk or mut 56/tk) resulted in a 71% and 57% reduction of the promoter activity respectively relative to 18-bp (+)/tk (Fig. 5B).

Correlation of DNA-Protein Complex I in EMSA with the Expression of 1.B Transcript

Since the activation of the tk promoter through the 18-bp or 26-bp DNA sequence was demonstrated to be cell-specific, we were interested to know whether there is any cell-specific DNA-protein interaction with the same DNA sequence and, if so, whether that correlates with the expression of 1.B. We assayed the NE prepared from five different cell lines (U1240MG, U1242MG, U251MG, U343MG, and CHII) of glial origin for the specific binding of the nuclear protein(s) with the DNA probe having the sequence −492 to −467 (Fig. 6). By RNase protection, it has been shown that U1240MG and U251MG are positive for 1.B transcript, whereas the remaining three cell lines are negative (26, 32). Fig. 6A showed that three distinct complexes (I, II, and III) were formed with U1240MG NE (lane 1) and these complexes were competed by a specific cold competitor (−489 to −467) (lane 2). Notably, the fastest mobility complex, complex I, is exclusively present in the 1.B-positive cell lines (U1240MG and U251MG) but not in the 1.B-negative cell lines (CHII, U343MG, and U1242MG) (Fig. 6, A and B). To understand whether there is any tissue-specific expression of similar factors, we also performed EMSA with the NE prepared from human brain and placenta (Fig. 6B). Interestingly, three specific DNA-protein complexes including complex I were also formed with the NE from the human brain (Fig. 6B, lane 5), but the nuclear proteins in the human placenta failed to form any of these complexes (lane 7). These results are consistent with the previous RNase protection studies, which showed the human brain tissue is positive for 1.B (26, 32) whereas placenta is negative for FGF-1 mRNA (5).

The DNA-Protein Complexes I, II, and III Share a Common DNA Binding Site

Since the methylation of guanine (G) residues at −482, −474, and −473 in the sense strand affected the formation of three complexes (I, II, and III) (Fig. 3), we were interested to
know whether these complexes result from the binding of nucleic factors with the DNA sequence $\text{2492 to 2467}$ at distinctly different site or an overlapping site. We tested each of these complexes in methylation interference assay and looked for the methylation of particular G residues that affect individual complex. Fig. 7 showed that each of these complexes preferably bound to the labeled and methylated DNA, which had no methylation of the G residues at $\text{2474 and 2473 in the sense strand}$. In addition, methylation of the G residue at $\text{2482}$ was also shown to interfere with complex II formation and to some degree with complex I formation. These results indicate that levels in the DNA-protein binding assay. A HindIII-EcoRI fragment containing the sequence $\text{2492 to 2467}$ was end-labeled and used as a probe. I, II, and III represent the three specific complexes formed with U1240MG NE. Panel B, the oligonucleotide (A, B, C, or D) was labeled by end-filling reaction with Klenow enzyme in the presence of $[\alpha-\text{32p}]dATP$. Equal count (30,000 cpm) of each oligonucleotide was incubated with 5 µg of U1240MG NE in the absence (−) or presence (+) of 50-fold molar excess of cold oligonucleotide A.
Brain-specific cis-Acting Element

DOC Treatment Has Little Effect on the Number of the Complexes Formed with U1240MG NE

Since complexes I, II, and III share a common DNA binding site, we addressed the question whether these three complexes result from protein-protein association of a common DNA-binding protein with non-DNA-binding proteins. For example, the DNA-binding protein E2F forms DNA-protein complexes of different mobilities in EMSA by associating with other proteins that are not directly associated with the DNA. In such a case, these complexes can be dissociated to a single complex in the same assay by prior treatment with DOC (37). We treated the NE with DOC to dissociate the protein-protein association of similar nature (if any) and assayed in EMSA to detect the number of specific DNA-protein complexes. Our results showed that DOC treatment did not abolish any of the three complexes, although their intensities were reduced in general (Fig. 9, lane 4). This observation implies that each of these three complexes results from the binding of DNA directly and that there are more than one specific DNA-binding protein recognizing the sequence −482 to −467.
Complex I Contains Two DNA-binding Proteins of 37 and 98 kDa

We used U1240MG NE and a photo affinity probe (as described under “Materials and Methods”) to analyze the DNA-protein complexes I, II and III by in situ UV cross-linking (Fig. 10). The bromo-dUTP-labeled photoaffinity probe also formed the same three specific complexes I, II, and III (data not shown) as formed by the regular probe (Fig. 6). We analyzed each of these complexes in denaturing polyacrylamide gels. Fig. 10 showed that complex I contains two DNA-binding proteins of apparent molecular masses of 37 and 98 kDa. Complex II contains a single protein of 98 kDa, and complex III has two proteins of 98 and 145 kDa. Based on the protein profile (Fig. 10) and the mobility of each complex in native gels (Fig. 6), it appears that complex I results from the heterodimerization of p37 and p98. Complex II may likely be a homodimer of p98 and complex III a heterodimer of p98 and p145 (Fig. 11).

DISCUSSION

The study of the tissue- or cell-specific distribution of FGF-1 transcripts has led to the identification of four different transcripts having the same protein coding exons but different 5'-untranslated exons (27–31). FGF-1B is the predominant transcript in brain, gliomas, and some glioblastoma cell lines (e.g. U1240MG and U251MG) (26, 32). Using U1240MG cell line, we have recently identified two regulatory regions, RR1
The apparent molecular masses of the DNA-binding proteins.

U1240MG was incubated with DOC followed by treatment with Nonidet P-40 and assayed for the DNA binding activity in the absence (−) or presence (+) of 50-fold molar excess of cold competitor (oligonucleotide B as described in Fig. 4A) using the same probe and conditions described in Fig. 4A. The final Nonidet P-40 concentration in the binding reaction (lanes 3–5) is 0.05%.

\[
\begin{align*}
\text{Complex I} & \quad \text{Complex II} \\
\text{Complex III} & \\
\end{align*}
\]

\[\text{ACGACCTGCTGTTTCCCTGGCAACTC}\]

**Fig. 9.** The specific complexes result from the binding of DNA with more than one specific DNA-binding protein. U1240MG NE was incubated with DOC followed by treatment with Nonidet P-40 and assayed for the DNA binding activity in the absence (−) or presence (+) of 50-fold molar excess of cold competitor (oligonucleotide B as described in Fig. 4A) using the same probe and conditions described in Fig. 4A. The final Nonidet P-40 concentration in the binding reaction (lanes 3–5) is 0.05%.

**Fig. 10.** Complex I contains two major DNA-binding proteins of apparent molecular masses of 37 and 98 kDa. The DNA-binding protein(s) labeled upon cross-linking with labeled photoaffinity probe (representing the sequence −484 to −467) from complex I, II, or III was separated in 10% SDS-polyacrylamide gel electrophoresis. The leftmost lane represents the sizes of the marker proteins. The arrows indicate the apparent molecular masses of the DNA-binding proteins.

\((-145 \text{ to } -114)\) and RR2 \((-507 \text{ to } -467)\), in the promoter of FGF-1 gene, which are important for FGF-1B expression (34). In the same study, we have also shown that the deletion of \(-490 \text{ to } -467\) sequence (Del-2) significantly reduced the reporter activity relative to the wild-type promoter \((-540 \text{ to } +31)\). In this study, we addressed the question whether the insertion of a tandem repeats of the same sequence into Del-2 in either orientation can functionally rescue the reporter activity above the wild-type level. As expected, the insertion of four tandem repeats in the sense orientation \((4U(+))\) restored the reporter activity above the wild-type level. However, the insertion of the same tandem repeats in the antisense orientation \((4U(-))\) had little effect on the reporter activity relative to Del-2. This result suggests that the sequence \(-492 \text{ to } -467\) positively regulates the FGF-1B promoter in an orientation-dependent manner. It is noted that other regulatory elements, such as the one for the platelet-derived growth factor B gene, are orientation-specific (38).

The region \(-540 \text{ to } -467\) has been shown previously (34) to be important for the enhanced activity of FGF-1B promoter. The DNase I footprinting experiment carried out in the present study with two overlapping genomic fragments \(-540 \text{ to } -357\), and \(-594 \text{ to } -357\) revealed that the nuclear protein(s) in U1240MG cells protects a common region \(-485 \text{ to } -467\) in both fragments. The methylation interference study as well as EMSA using overlapping oligonucleotides also established that the minimal region for the DNA-protein interaction resides at \(-484 \text{ to } -467\). It is noted that mutation at \(-484, -478, -472\) (mut 123), which disrupts the formation of DNA-protein complexes, also reduces the transcriptional activity of the reporter activity by 76% in the context of \(-540 \text{ promoter of FGF-1B} (34). These results indicate that \(-484 \text{ to } -467\) is an essential element contributing to the promoter activation through the DNA-protein interaction.

To further test the above prediction, we determined the activity of the minimal tk promoter in the absence or presence of the 18-bp \((-484 \text{ to } -467)\) or 26-bp \((-492 \text{ to } -467)\) sequence in U1240MG or U1242MG cells. It was demonstrated that both sequences enhanced the reporter activity driven by the heterogeneous tk promoter to a similar extent in U1240MG. The data indicated that the 18-bp DNA sequence, which is sufficient for DNA-protein interaction, is also sufficient to enhance activity of the tk promoter. This enhancer activity is cell-specific, since none of these two chimeric promoter-reporter constructs enhanced the tk promoter activity when transfected into U1242MG cells. By contrast, the full-length tk construct (tk\((-200)) activated the reporter activity to the similar extent in both cell lines. The activation of tk promoter in U1240MG cells through the 18-bp element is also site-specific because the mutant oligonucleotides (mut 456 or mut 56), which are unable to form specific DNA-protein complex, failed to enhance the activity of tk promoter. Thus, the 18-bp cis-acting sequence

**Fig. 11.** A model of DNA-protein interactions in RR2 of the FGF-1B promoter. DNA-protein interactions occurred in complexes I, II, and III are diagrammed. The p37<sup>pe</sup> protein was detected both in a Southwestern analysis using oligonucleotide \(-489 \text{ to } -467\) as a probe (34) and in complex I (Fig. 10). The p98 protein was detected in all three complexes, while p145 was detected only in complex III. We hypothesize that it is this concerted interaction among the three different proteins and the RR2 that regulates brain-specific transcription of the 1.B. Asterisks indicate the three guanine residues in the antisense strand that directly contact the nuclear factors. Only the sense strand of FGF-1B promoter is shown. The arrows indicate the major transcriptional activation start site for 1.B. The underlined sequence is identical to the corresponding mouse FGF-1B promoter sequence.
functions not only in the context of the native FGF-1.B promoter (34) but also in the context of the heterologous tk promoter (Fig. 5). The orientation dependence of this 18-bp sequence is, however, unconventional but consistent with the data shown in Fig. 1. Moreover, these results also indicate the presence of cell-specific factor(s) interacting with the 18-bp sequence is important for the enhancement of the promoter activity.

EMSA revealed that complex I is unique when using the NE of U1240MG and U251MG cells; both cell lines are known to express 1.B (26, 32). The absence of complex I when using the NE of U1242MG or other 1.B-negative cells supports the view that nuclear factor(s) allowing the formation of complex I may be important for the activation of FGF-1.B promoter. Interestingly, the NE from the human brain tissue also formed three specific DNA-protein complexes including complex I, and this tissue predominantly expresses 1.B (26, 32). The human placenta is known to be negative for FGF-1 mRNA (5), and the NE prepared from this tissue failed to show any of these complexes. These data suggest tissue- or cell-specific expression of nuclear factor(s) may be important for the tissue- or cell-specific expression of 1.B.

Among the three complexes (I, II, and III), since complex I appears to be linked with 1.B expression, we attempted to precisely locate the binding site that is crucial for this complex formation. The methylation interference assay using the bound probes eluted from each individual complex revealed that complexes I, II, and III resulted from the contact of nuclear factor(s) with G residues at −474 and −473 in the sense strand. In addition, complex II has also been found to contact with the G residue at −482 in the same strand. EMSA using oligonucleotide with mutation at −482, −474 and −473 (mut 456) or at −474 and −473 (mut 56) also shows that the formation of each of these complexes is affected to a similar extent in both cases. Furthermore, EMSA using the oligonucleotide (−489 to −467) with mutation at different sites (−484, −478, and −472) also failed to differentiate these three complexes in terms of their specific binding sites and showed that the G residue at −478 is crucial for the formation of these complexes. These results suggest that these complexes share a common DNA binding site including the sequence −478 to −473.

DNA-protein complexes with different mobilities may be formed by the interaction of multiple DNA-binding proteins of different sizes to a common binding site (39) or by the association of DNA-binding protein with other proteins that do not directly bind the DNA (35). Our results showed that more than one DNA-binding protein binds to the sequence −484 to −467. In situ UV cross-linking study of these three complexes reveals that complex I contains two DNA-binding proteins of 37 and 98 kDa, complex II contains a single DNA-binding protein of 98 kDa, and complex III contains two DNA-binding proteins of 98 and 145 kDa. Based on the protein profile and the mobility of each complex in native gels, it is most likely that complex I results from heterodimerization of p37 and p98. Complexes II and III may likely be a homodimer of p98 and a heterodimer of p98 and p145, respectively. These results suggest that formation of the activating complex in the FGF-1.B promoter requires both p37 and p98. Remarkably, a DNA-binding protein of 37 kDa has been shown to be present in human brain tissue and U1240MG cells (but not in U1242MG cells) that can specifically bind to the sequence −489 to −467 in Southwestern analysis (34). In EMSA, however, we could not detect the specific binding of a 37-kDa protein in U1240MG NE that can alone bind the same DNA sequence. This discrepancy may be due to the variation of the assay conditions used to study the DNA-protein interaction. It is pertinent to mention that a specific faster mobility complex (faster than complex I) was demonstrated in EMSA in the same cell line when 1 μg or less than 1 μg of poly(dI-dC) was used as nonspecific competitor (data not shown). Moreover, it is significant that a DNA-binding protein of the same size (i.e. 37 kDa) from the same source (i.e. U1240MG cells) and the same binding specificity (34) is present in complex I, which is also unique for 1.B-positive cells or tissues.

Our data, taken together, allow us to propose a testable model (Fig. 11) for the DNA-protein interactions at the RR2 (precisely at positions −484 to −467) that may explain the cell line-specific activation of FGF-1.B. Based on the present study, it is likely that a 98-kDa protein binds to a minimal DNA sequence −484 to −467 as a homodimer. Two other DNA-binding proteins of 37 and 145 kDa bind to the 98-kDa protein, forming two heterodimers of different mobilities. Based on the finding that complex I is present exclusively in 1.B-positive cells (U1240MG, U251MG, and in human brain), we hypothesize that the expression of the p37 followed by its heterodimerization with p98 resulting in the formation of complex I is a prerequisite for the enhancer activity of RR2. Whether the FGF-1.B promoter commits itself to activation in a given cell may depend on the stoichiometric amounts of p37, p98, and p145. It is likely that the availability of p37 is crucial for the expression of 1.B. Since the identified cis-acting sequence shows no similarity with sequences recognized by any other known transcription factors (GenBank, October 1996 release), it is most likely that p37 represents a novel transcription factor, which we tentatively designated as p37brn. Whether p98/p98 homodimer and p98/p145 heterodimer compete with p37/p98 heterodimer for the common binding site and thereby neutralizing the positive trans-acting effect of the latter remains to be determined. As a corroboration to the model proposed here, we have cloned the mouse FGF-1.B promoter (33) and showed that the sequences most crucial for binding to p37brn (between nucleotides −483 and −473) are identical and positioned in the same context relative to the transcription start sites between the two species (33). Isolation of the cDNA expressing these DNA-binding proteins including p37brn, either by screening the expression library using the minimal binding sequence or by protein purification, will help us verify the above model and study the regulation of 1.B expression in brain as well as in glioblastoma.

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