Activation of a Nuclear DNA-binding Protein Recognized by a Transcriptional Element, bcn-1, from the Laminin B2 Chain Gene Promoter*

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Laminin is a major component of the glomerular basement membrane (1). It is a large non-collagenous glycoprotein composed of three chains connected by an α-helical coiled-coil domain (2). At least five distinct chains of laminin are known to exist. The basement membrane contains only a unique set of laminin chain heterotrimers; they all contain B2 chain in a combination with either A chain or merosin and either B1 or 5 chain (3–5). In the kidney, the expression of the variant laminin chains depends on the tissue type (6), stage of development (7–9), and disease (10). The level and distribution of laminin in the glomerular basement membrane also varies with types of glomerular disease (11, 12). Although it is known that an abnormal accumulation of laminin chains contributes to the progression of glomerular scarring, the molecular mechanisms responsible for the increased synthesis of laminin are very poorly understood.

In the kidney, laminin is thought to be synthesized by both glomerular epithelial and mesangial cells (3, 13). We have demonstrated previously that treatment of glomerular epithelial cells with IL-1β1 induced a transient increase in laminin B2 chain mRNA levels (14). This increase was associated with the activation of NF-κB DNA binding activity recognized by a κB-like motif contained in the murine laminin B2 chain gene promoter (14). This association suggests that production of laminin chains in glomerular cells could, in part, be transcriptionally mediated.

Promoters of a number of the laminin chain genes share similarities, suggesting that transcription of laminin chain genes is regulated by similar or overlapping mechanisms. Human and murine B1 and B2 chain promoters have no TATA or CAAT boxes, contain a number of GC boxes, κB consensus sequences, and potential Sp1 binding sites (GGCGCG) (15–19). TATA-less promoters typically contain a number of Sp1 DNA-binding sites, and the Sp1 transcription factor is thought to play a particularly key role in the initiation of transcription of genes that contain no TATA boxes in their promoters. The human B2 promoter contains a potential AP-2 binding site (CCCCAGGC) (15, 17), suggesting that this or related factor along with NF-κB and Sp1 might play a role in the transcriptional control of expression of laminin genes. To gain more insight into the transcriptional regulation of laminin genes, we used a computer-based analysis to compared the sequences of the murine and the human laminin B2 chain gene promoters (17, 18). Here, we demonstrate that in mesangial cells IL-1β, PMA-, and TGF-β- induced transient increase in laminin B2 chain mRNA levels is preceded by the induction of nuclear DNA binding activity recognized by a highly conserved enhancer element, designated bcn-1, contained within both the rodent and the human laminin B2 chain gene promoter.

MATERIALS AND METHODS

Mesangial Cell Culture—The rat mesangial cell line was established from collagenase-treated glomeruli, and cells were characterized as described previously (20). Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (Life Technologies, Inc.), 100 U/ml penicillin, 100 μg/ml streptomycin, and 200 μg/ml L-glutamine. Cells were maintained at 37°C in 5% CO₂ in air and were passaged every 5–7 days by scraping.

Reagents—N-[N-[(3-trans-Carboxyoxirane-2-carbonyl)-l-leucyl]agmatine and antipain dihydrochloride was purchased from Boehringer Mannheim, leupeptin and PMSF from Sigma.

Northern Blot Analyses—Total RNA was extracted essentially as described previously (21) with some modifications. Briefly, after treatment of mesangial cells (approximately 1.0× 10⁷ cells) with inducing agents, cells were directly lysed and denatured in 2.0 ml of RNAzol B™ (Cinna Scientific Inc., Friendswood, TX) to isolate RNA. RNA was analyzed as described previously (22). A total of 15 μg of RNA was analyzed by electrophoresis on 1% agarose gels.

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1The abbreviations used are: IL-1β, interleukin-1β; PMA, phorbol 12-myristate 13-acetate; TGF-β, transforming growth factor-β; PMSF, phenylmethylsulfonyl fluoride; MOPS, 4-morpholinepropanesulfonic acid.
For site-directed mutagenesis of the bcn-1 motif, the rat laminin B2 chain gene promoter (–948 to –79 relative to the transcription initiation site) was subcloned into a luciferase reporter gene, pGL3 enhancer (Promega, Madison, WI). Mutagenesis of the five base pairs (see bold below) required for protein binding was performed using Quick-Change™ site-directed mutagenesis kit (Stratagene, La Jolla, CA) with oligonucleotides mut-bcn-1-BAMHI sense 5'-CCACGCGCCCTGGATCTCCTGGCGCTTCCCTCC-3' and antisense 5'-GGGAAGGGCGCAGGATCACCAGGCGCCGTTGG-3'. The mutation was verified by restriction analysis (a new BamHI site was created by the mutagenesis) and by direct dideoxynucleotide sequencing with Sequenase (U. S. Biochemical Corp.).

Transient Transfection of Mesangial Cells and Luciferase Reporter Gene Assay—Mesangial cells were transiently transfected using a lipidation method (29). Briefly, dioleoyl-

For Northern Blot Analysis of the Laminin B2 Chain mRNA Levels in Mesangial Cells Treated with Cytokines—In the glomerulus, laminin is a major constituent of the lamina rara externa of the glomerular basement membrane (13). Although the mechanisms responsible for the regulation of laminin chain synthesis in the glomerulus are poorly understood, laminins are thought to be produced by both the mesangial and glomerular epithelial cells. To elucidate the potential mechanisms that are responsible for the regulation of laminin production in glomerular cells, we examined the expression of laminin B2 chain mRNA levels in response to treatment of mesangial cells with a number of known cytokines and mitogens. Fig. 1 illustrates an autoradiograph of a Northern blot of total RNA isolated from untreated (Control, lane 1) and treated (lanes 2–10) mesangial cells probed with either 32P-labeled murine laminin B2 chain cDNA (4) (upper panel) or 32P-labeled 28 S probe (lower panel) as a loading control. TGF-β (lanes 2–4), IL-1β (lanes 5–7), and PMA (lanes 8–10) induced a transient increase

Plasmid Constructs—Synthetic bcn-1 oligonucleotide was used to construct reporter plasmid containing a dimer of wild-type and mutated bcn-1 oligonucleotides. The bcn-1 dimer inserts were subcloned in the BglII and MluI sites of pGL3 control vector (Promega, Madison, WI), and the nucleotide sequence of the inserts was confirmed by dideoxy sequencing with Sequenase (U. S. Biochemical Corp.) (28).

RESULTS

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4 h of stimulation (compare lane 1 to lanes 3, 6, and 9 in the upper panel), and after 6 h of treatment the mRNA levels returned to baseline (compare lane 1 to lanes 4, 7, and 10 in the upper panel). In contrast to the laminin B2 chain message, the levels of 28 S RNA did not increase (lanes 1-10 in the lower panel) in response to treatment with these agents. These results are analogous to the IL-1β effects seen in glomerular epithelial cells, where IL-1β also induced a transient increase in laminin B2 chain mRNA levels but the peak effect was seen after 2 h of treatment (14).

A Motif from the Laminin B2 Chain Gene Promoter Recognizes an Inducible DNA Binding Activity in Nuclear Extracts from Mesangial Cells—in many cell systems PMA, IL-1β, and TGF-β increase message levels through transcriptional mechanisms (32–34). Hence the transient increase in laminin B2 chain mRNA levels in mesangial (Fig. 1) and glomerular epithelial cells (14) might, likewise, be transcriptionally mediated. To identify transcriptional elements contained within the laminin B2 chain gene promoter, we compared the human (17) and mouse (18) sequences 5' to the first exon using the GCG program (Genetics Computer Group, Madison, WI). Although the overall promoter sequences in the two species are significantly divergent, there was a region in the human promoter (−550 to −525 relative to the first exon) that contained sequences identical or nearly identical to short regions (9–17 nucleotides long) present in the murine laminin B2 chain gene promoter (Fig. 2). We reasoned that one or more of these similar promoter motifs might have been evolutionarily conserved in order to recognize the same or related transcription factors in rodents and humans. To test this hypothesis, we synthesized a double-stranded oligonucleotide that represented the human laminin B2 chain gene promoter sequence. To take into account the two shorter murine fragments that were disparate from the human sequence at a single point, the synthetic oligonucleotide was programmed degenerate to contain either G or T at position −546, and either A or T at position −543 (human laminin B2 chain gene promoter) (17). Fig. 3. illustrates an autoradiograph of a gel shift assay performed on nuclear extracts from untreated and treated rat mesangial cells using as a probe the 32P-labeled, conserved double-stranded oligonucleotide motifs, 5'-CCC(G/T)CCC(A/T)CCCTCGGCCGCCCCGCTCC-3' (sense strand) and 5'-CTCGCGCCGCCCCCTCCC-3'. Treatment of mesangial cells with 10−7 M PMA (Fig. 3A) induced a transient increase in DNA binding activity recognized by this 32P-labeled probe in nuclear extracts from these cells. This motif is designated bcn-1, and its cognate protein (or protein complex) is termed BCN-1. The nuclear BCN-1 DNA binding activity peaked after 1 h of treatment with PMA (compare lane 1 to lane 2), decreased slightly after 2 h (compare lane
Fig. 4. Competition for DNA binding of nuclear proteins between xB and bcn-1 motifs. Nuclear proteins extracted from PMA-treated mesangial cells were analyzed by gel shift assay using 32P-labeled bcn-1 (panel A) or xB (panel B) probes in absence (control) or presence of 5-, 50-, or 500-fold molar excess of the unlabeled oligonucleotide competitor. Unlabeled (cold) synthetic double-stranded oligonucleotides containing either the wild-type or mutant xB elements, or the bcn-1 motif were used as competitors. Unbound (Free) DNA and relevant bcn-1-protein and xB-protein complexes are marked by arrows.

The interaction of the bcn-1 Motif with Its Cognate Protein(s) Is DNA Sequence-specific—To examine whether binding of the bcn-1 protein to the bcn-1 probe is DNA sequence-specific, nuclear proteins extracted from PMA-treated mesangial cells were incubated with the 32P-labeled bcn-1 probe in the absence (control, lane 1) or presence of increasing (lanes 2–10) concentrations of unlabeled competitor (or to analysis of DNA binding in a gel shift assay. The autoradiograph of the gel (Fig. 4A) demonstrates that binding to the 32P-labeled bcn-1 motif was effectively competed by an excess unlabeled bcn-1 DNA (compare lanes 6 and 7 to lane 1) but not by a comparable excess of oligonucleotide bearing either the wild-type or mutated xB motif (compare lanes 4 and 10 to lane 1). As a control for these experiments, we used the same nuclear extracts from PMA treated mesangial cells but instead tested DNA binding to 32P-labeled synthetic oligonucleotide containing the wild-type xB (Fig. 4B) (24), alone or in the presence of increasing excess of oligonucleotides containing either the wild-type or mutated xB (24), or bcn-1 motif. Treatment of mesangial cells with PMA activates NF-xB (Fig. 8). An autoradiograph (Fig. 4B) from these competition experiments shows that the binding to the 32P-labeled NF-xB probe was effectively competed by an excess of an unlabeled oligonucleotide containing the wild-type xB motif (compare lanes 3 and 4 to lane 1), but not by a comparable amount of oligonucleotide containing either the mutated xB or the bcn-1 motif (compare lanes 6, 7, 9, and 10 to lane 1). This series of experiments demonstrated that the bcn-1 motif recognizes its cognate nuclear protein (or protein complex) in a DNA sequence-specific manner and that the bcn-1 DNA-binding protein is not the transcription factor NF-xB.

The (A/T)CCT Nucleotide Region Contained within the bcn-1 Motif Is Required for Recognition of the Cognate Protein—To define the nucleotide bases contained within the bcn-1 motif that mediate protein binding, we designed “mutated” bcn-1 oligonucleotides and tested their ability to recognize nuclear proteins from PMA-treated mesangial cells. Prior to synthesis and their use in gel shift assays, the designed mutated bcn-1 sequences were rechecked against sequences in the transcription data base in GCG (Genetics Computer Group, Madison, WI) to ensure that a known transcription factor DNA recognition site was not created. An autoradiograph of a gel shift assay of nuclear proteins extracted from PMA-treated mesangial cells using these 32P-labeled double-stranded oligonucleotides and their nucleotide sequences are shown in Fig. 5. Mutant bcn-1A (5'-CCCC-3' motif in the coding strand (sense) located in the 3' half of bcn-1 was converted to 5'-ATTA-3' motif) recognized PMA-inducible nuclear DNA binding activity with kinetics and electrophoretic mobility indistinguishable from the nuclear DNA binding activity recognized by the wild-type bcn-1 motif (compare lanes 4–6 to lanes 1–3). In sharp contrast, a 32P-labeled mutant bcn-1B (5’-(A/T)CCT-3’ motif in the coding strand) did not recognize any inducible nuclear DNA binding activity (compare lanes 7–9 to lanes 1–3). These gel shift assays indicate that the (A/T)CCT nucleotide stretch in the middle of the bcn-1 motif is required for the recognition of the nuclear PMA-inducible protein (or protein complex).

A Single Nucleotide Mutation in the bcn-1 Motif Disrupts Protein Binding—To maximize the likelihood of identification of a inducible nuclear factor, we have initially used a preparation of degenerate synthetic oligonucleotides containing sequences that were degenerate in two positions; one position was programed to contain either G or T, and another position contained either A or T. Thus, this mixture was made up of four different sequences differing from each other by one or two nucleotide bases. To determine which sequence(s) in the degenerate mixture is responsible for the binding of the BCI-1 protein, we separately synthesized double-stranded oligonucleotides representing one of the four possible bcn-1 sequences. A gel shift assay (Fig. 6) demonstrated that the oligonucleotide representing exactly the human laminin B2 promoter sequences (~550 to ~525) (Fig. 2) (17) (lanes 1–3) recognized a very strong PMA-inducible DNA binding activity. In a sharp contrast, the other three oligonucleotides either did not bind it at all (compare lanes 1–3 to lanes 4–9) or the binding was very weak (lanes 10–12). The observation that a single nucleotide base pair replacement in this promoter region was sufficient to...
block binding of the inducible factor indicates that the recognition of the bcn-1 nucleotide motif by the BCN-1 protein is remarkably specific. This and the previous set of experiments allow us to approximately define the 5'-GGGCAGGGCCCTCAAACCTCTGGC-3' region and identify both G and A (shown in bold) as obligatory for protein binding. An identical sequence is also present in the mouse (Fig. 2) (18) and the rat laminin B2 chain gene promoter. 

To determine whether or not the bcn-1 motif contains a transcriptional element that recognizes a known transcriptional factor, we compared the bcn-1 motif with DNA elements that this motif contains a transcriptionally active element from the apoE B1 gene promoter, 5'-CCC-3'. It drives gene expression by binding to GC-rich transcriptional factor that plays an important role in transcriptional regulation of expression of many housekeeping genes (19). It drives gene expression by binding to GC-rich promoter nucleotide sequences (36). Because the bcn-1 motif is also GC-rich (Fig. 2), we next tested whether or not the bcn-1-binding protein is in fact Sp1. To do that, nuclear extracts from PMA-treated cells were preincubated with either anti-Sp1 antibody or an excess of unlabeled oligonucleotide containing the Sp1-binding site prior to binding to the 32P-labeled bcn-1 probe. The DNA-protein complexes were then resolved by gel shift assay as in Fig. 7. The autoradiograph from this experiment (Fig. 7A) demonstrates that the anti-Sp1 antibody did not supershift or alter in any other way the electrophoretic mobility of the bcn-1-protein complex (compare lanes 2 and 3). This suggests that the protein recognized by the bcn-1 motif is not Sp1. An excess of unlabeled Sp1-binding DNA did not have an effect on the intensity of the bcn-1-BCN-1 complex, further showing that the bcn-1 motif does not recognize the Sp1 transcription factor (compare lanes 2 and 3). As a positive control for these experiments, we concurrently performed gel shift assay on the same nuclear extracts using a 32P-labeled synthetic oligonucleotide containing the Sp1-binding site as the probe. Although not as dramatic as with the BCN-1 response, treatment of mesangial cells with PMA increased nuclear levels of Sp1 (Fig. 7, compare lanes 1 and 2 in panel A to those in panel B). The fact that the DNA-protein complexes shown here (Fig. 7B) contain Sp1 is demonstrated by the supershift seen when the nuclear extracts were preincubated with the anti-Sp1 antibody (compare lanes 2 and 3), and by the observation that unlabeled synthetic oligonucleotide containing the Sp1-binding site markedly diminished the intensity of the 32P-labeled DNA-protein complex (compare lanes 2 and 4). Collectively, these results demonstrate that, although bcn-1 is a GC-rich motif, it does not recognize the transcriptional factor, Sp1.

The protein synthesis inhibitor cycloheximide blocks the induction of the BCN-1 DNA binding activity—To determine whether new protein synthesis is needed for the activation of the BCN-1 DNA binding activity, mesangial cells were treated

**Fig. 5. Comparison of DNA binding of nuclear proteins to oligonucleotides containing wild-type and mutated bcn-1 motif.** DNA binding activity of nuclear proteins was compared in a gel shift assay using 32P-labeled double-stranded oligonucleotides containing either the wild-type or mutated bcn-1 motifs. The sequences of the sense strands are shown in the lower panel. Nucleotides that were converted from the wild-type to the mutated bcn-1 oligonucleotides are shown in bold. The autorograph shows gel shift by nuclear proteins from a time course of PMA-treated (10^-7 M) mesangial cells assessed with the wild-type (lanes 1–3), mutant A (lanes 4–6), and mutant B (lanes 7–9) bcn-1 oligonucleotides.

**Fig. 6. Comparison of DNA binding of nuclear proteins to the wild-type bcn-1 motif and bcn-1 motifs containing single or double nucleotide substitution.** DNA binding activity of nuclear proteins was compared in a gel shift assay using 32P-labeled double-stranded oligonucleotides containing either the wild-type or mutated bcn-1 motifs. The sequences of the sense strands are shown in the lower panel. The mutated nucleotides are shown in bold. The autorograph shows gel shift by nuclear proteins from a time course of PMA-treated (10^-7 M) mesangial cells assessed with the wild-type (lanes 1–3), and bcn-1 containing a single (lanes 4–6) or double nucleotide base pair substitution mutant B (lanes 7–9) bcn-1.
Either within the context of the laminin B2 chain promoter or when cloned upstream of heterologous promoter—To assess the transcriptional activity of the bcn-1 motif, we cloned a synthetic oligonucleotide containing either a wild-type or a mutant type bcn-1 dimer motif into a pGL3-control vector bearing the luciferase reporter gene (Promega, Madison, WI). Luciferase activity of the pGL3-control vector containing the wild-type bcn-1 dimer transiently expressed in untreated mesangial cells was 97.4 ± 13.6% (Fig. 9A, lane 1) in untreated cells and increased to 174.4 ± 12.9% (lane 2) after 24 h of treatment of cells with PMA (n = 6, p < 0.005). Relative luciferase activity of the pGL3-control vector containing the mutant-type bcn-1 dimer in untreated cells was 34.2 ± 11.3% (Fig. 9, lane 3) and 38.2 ± 2.9% (lane 4) after 24 h of PMA stimulation, a 4% difference that was not statistically significant (n = 6, p = 0.73).

To evaluate the transcriptional activity of the bcn-1 motif within the context of the laminin B2 promoter, the −948 to −78 (relative to the transcription initiation site) fragment of the rat laminin B2 chain promoter containing the bcn-1 motif was cloned upstream of a luciferase reporter gene. Mutagenesis of the five bcn-1 bases, that are required for protein binding was performed using sense and antisense synthetic oligonucleotides that were designed to contain mutated bcn-1 motif and a BamHI site (see "Materials and Methods"). Relative luciferase activity of the pGL3-enhancer vector containing the wild-type laminin B2 chain promoter transiently expressed in mesangial cells was 107.5 ± 24.2% (Fig. 9B, lane 1) and increased to 236.3 ± 29.7% (lane 2) (n = 4, p < 0.001) after 24 h of PMA stimulation. Luciferase activity of the pGL3-enhancer vector containing the laminin B2 chain promoter with a mutated bcn-1 site, was 89.4 ± 7.1% (lane 3) in untreated cells and 125.0 ± 14.2% (lane 4) after 24 h of treatment of cells with PMA, an increase that was not statistically significant (n = 4, p = 0.25). These results demonstrate that the bcn-1 motif from the laminin B2 chain gene promoter is transcriptionally active and PMA-responsive. The wild-type laminin B2 promoter was only weakly responsive to IL-1β treatment, and the small response was not blocked by mutating the bcn-1 motif (data not shown). While the activation of the bcn-1 DNA binding activity by PMA in mesangial cells has consistently been very robust, the level of BCN-1 activation by IL-1β has not been as large (Fig. 3, compare panel

**FIG. 7.** Comparison of DNA binding of nuclear extracts to the bcn-1 motif and Sp1-binding nucleotide consensus sequence. Nuclear proteins (30 μg/reaction) extracted from PMA-treated mesangial cells were analyzed by gel shift assay using 32P-labeled double-stranded oligonucleotides. Panel A, DNA-binding reaction with 32P-labeled bcn-1 probe was carried out in a standard way (lane 2) or in the presence of either 1 μl of anti-Sp1 antibody (lane 3) or a 200-fold molar excess of synthetic double-stranded oligonucleotide containing the Sp1-binding site (lane 4). Panel B, DNA-binding reaction with 32P-labeled Sp1-binding probe was carried out in a standard way (lane 2) or in the presence of either 1 μl of anti-Sp1 antibody (lane 3) or a 200-fold molar excess of synthetic double-stranded oligonucleotide containing the Sp1-binding site (lane 4). Binding to the 32P-labeled Sp1-binding probe in absence (each lane 2), or presence of Sp1 antibody (each lane 3) or 200-fold molar excess of the unlabeled competitor (each lane 4). DNA-protein complexes were resolved by gel electrophoresis and gels were autoradiographed as before (Fig. 3).

for 1 h with 25 μg/ml cycloheximide, a protein synthesis inhibitor (37), prior to treatment with PMA. At given time points cells were harvested, nuclei were isolated, and nuclear protein extracts were assayed in a gel shift assay using either 32P-labeled bcn-1 (panel A) or NF-κB (panel B) probes. Unbound (free DNA and relevant bcn-1 and κB-protein complexes are marked by arrows.

**FIG. 8.** Effects of the protein synthesis inhibitor, cycloheximide, on the activation of BCN-1 (Panel A) and NF-κB (Panel B) DNA binding activity. Mesangial cells were either untreated (lanes 1–3) or were pretreated for 1 h with 25 μg/ml cycloheximide (CHX) (lanes 4–6), followed by stimulation with PMA (10−7 M). At given time points, cells were harvested, nuclei were isolated, and nuclear extracts were prepared. Nuclear proteins were analyzed by gel shift assay using either 32P-labeled bcn-1 (panel A) or NF-κB (panel B) probes. Unbound (free DNA and relevant bcn-1 and κB-protein complexes are marked by arrows.
A to panel B). Therefore, the discrepancy between the measured IL-1β-induced BCN-1 DNA binding and the reporter gene activity in transient transfections may be accounted for by the fact that the gel shift analysis is more sensitive. Also of note is the observation that at 2 h of IL-1β the BCN-1 DNA binding activity is nearly gone, while the laminin B2 chain mRNA does not peak until 4 h. These discrepancies may reflect involvement of multiple factors in the IL-1β-induced gene expression.

**DISCUSSION**

In vitro studies have demonstrated that glomerular mesangial and epithelial cells are the likely source of laminin chains in the glomerulus (13). Both of these glomerular cell types have been successfully grown in culture (22, 40), providing useful model systems to examine mechanisms that regulate laminin chain synthesis. A number of growth factors activate glomerular mesangial and epithelial cells, including IL-1 and TGF-β (41), and some of these agents have previously been reported to stimulate laminin production in these cells (42, 43). We have shown previously that IL-1β increases laminin B2 chain mRNA levels in cultured glomerular epithelial cells (14). In the present study, we have extended these observations by demonstrating that treatment of mesangial cells with either PMA, IL-1β, or TGF-β also results in a transient rise in laminin B2 chain mRNA levels (Fig. 1). The growth factor-induced increase in laminin B2 chain gene expression in glomerular mesangial (Fig. 1) and epithelial (14) cells is likely to be transcriptionally mediated.

A number of methods have been used to identify transcriptional elements. The traditional method consists of deletion analysis of gene promoters using reporter genes such as chloramphenicol acetyltransferase (44) or luciferase (30) as a read-out for transcriptional activity. This approach resulted in the identification of most of the known transcription factors. The present study illustrates application of an alternative strategy to search for promoter motifs that recognize inducible proteins. This strategy is based on the fact that even in related species the non-coding DNA regions have low overall sequence homology, but in order to recognize homologous transcription factors, the regulatory DNA elements contained in the untranslated regions are very likely to be highly conserved across related species. The conserved regulatory motifs are simply identified by computer-driven alignment of the promoter sequences from different species. The promoter sequences of regions that are conserved are then analyzed against transcription databases to determine (i) whether or not other gene promoters contain these motifs, (ii) whether or not these motifs are known to be transcriptionally active, and (iii) whether or not these or homologous nucleotide motifs are known to recognize transcription factor(s). Based on the computer database search, one can then choose which of the conserved promoter motifs ought to be used to design synthetic oligonucleotides to test if they recognize inducible DNA binding activities in gel shift assays. The advantage of this strategy is its simplicity because it is based on computer analysis and gel shift assays. This strategy is particularly suitable for analysis of those gene promoters whose nucleotide sequences in related species have an overall...
low sequence homology but contain short (20–30 nucleotides) regions that are conserved.

We applied this strategy to begin identification of transcription factors that potentially control laminin B2 chain gene expression. While the coding nucleotide sequences of the mouse and human laminin B2 chain genes share high homology, the region 5' to the first exons are overall divergent (4, 17, 18, 45, 46). We identified highly conserved nucleotide regions and showed that one of the conserved motifs, termed bcn-1 (Fig. 2), did recognize an inducible nuclear protein(s), BCN-1 (Fig. 3). We demonstrated that a single nucleotide base pair replacement was sufficient to block protein binding (Fig. 5), indicating that this interaction is highly specific. The BCN-1 DNA binding activity is activated by the same growth factors (Fig. 3) that increase laminin B2 chain mRNA levels (Fig. 1). This association suggests that the BCN-1 factor might contribute to the induction of laminin B2 chain gene expression by growth factors.

As with Sp1-binding DNA (Fig. 8), the bcn-1 motif remains to be identified, this suggests that the BCN-1 factor might contribute to the induction of laminin B2 chain gene expression by growth factors.

In summary, a simple strategy was applied to identify an inducible nuclear DNA binding activity recognized by a conserved motif from the laminin B2 chain gene promoter. In the context of the laminin B2 chain gene promoter, the bcn-1 motif is transcriptionally active and PMA-inducible, suggesting that it plays a role in the induction of the laminin B2 chain gene transcription in mesangial cells. Work is in progress to determine the cDNA encoding this protein.

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