Here we characterize a novel murine nuclear protein, which we named NBP-45, that is related to the ubiquitous nuclear proteins HMG-14/-17, binds specifically to nucleosome core particles, and can function as a transcriptional activator. NBP-45 mRNA is expressed at low levels and in variable amounts in all mouse tissues tested but is especially abundant in RNA extracted from 7-day-old mouse embryos, suggesting that it functions in early embryonic development. NBP-45 is composed of 406 amino acids and is encoded by a single size transcript. The region spanning the N-terminal 85 amino acids contains three segments that are highly homologous to functionally important domains in the HMG-14/-17 protein family: the nuclear localization signal, the nucleosome binding domain, and the chromatin unfolding domain. The protein region spanning the C-terminal 321 amino acids has a 42% content of negatively charged residues. The first 23 amino acids contain a region necessary for nuclear entry of the protein, the region spanning residues 12–40 is the main nucleosomal binding domain of the protein, and the negatively charged, C-terminal domain is necessary for transcription activation. The functional domains of NBP-45 are indicative of a nuclear protein that binds to nucleosomes, thereby creating a chromatin region of high local negative charge. Our studies establish the nucleosomal binding domain as a protein motif that is present in other than just the ubiquitous HMG-14/-17 proteins. We suggest that the nucleosomal binding domain motif is a protein module that facilitates binding to nucleosomes in chromatin.

In the cell nucleus, the orderly progression of many DNA-related activities, such as transcription, replication, recombination, and repair, are associated with changes in the higher order structure of the chromatin fiber and with a temporal disassembly of the nucleosome. These structural changes are facilitated by multiple types of reversible posttranslational modifications of the histones and by the activities of various multiprotein complexes that disrupt the histone-DNA interactions in nucleosomes (1–9). In addition, structural proteins that lack known enzymatic activity, such as the high mobility group (HMG)1 proteins, are also known to modify the structure of their DNA binding site and induce an architecture that facilitates and enhances various DNA-related activities (10, 11).

The HMG protein family is subdivided into three subfamilies: the HMG-1/-2 subfamily, the HMG-I/Y subfamily, and the HMG-14/-17 subfamily. Each of these subfamilies has a unique protein signature and a distinct functional motif. The HMG-1 domain is the functional domain of the HMG-1/-2 subfamily, the AT-hook is the functional domain of the HMG-I/Y family, and the nucleosomal binding domain is the functional motif of the HMG-14/-17 proteins. Through these domains the HMG proteins bind to their DNA or chromatin target, with little if any specificity for the underlying DNA sequence (10, 11).

Two of the HMG functional domains, the HMG-1, and the AT-hook motifs have been identified as embedded in numerous nuclear proteins that interact with DNA in a sequence specific manner (10, 12, 13). These motifs are major sites of interaction between the proteins and their specific binding sites. The third HMG motif, the HMG-14/-17 nucleosomal binding domain, seems to be much less prevalent (10, 11) and has been detected by yeast two-hybrid system, only in Trip-7, isolated as a cDNA clone from a HeLa cell cDNA library (14). Although the interaction of the translation product of this clone with nucleosomes has not been yet described, the high degree of sequence homology with HMG-14/-17 proteins suggest that Trip-7 will interact with chromatin subunits.

Here we report the isolation and characterization of a novel protein, which we named NBP-45 (nucleosomal binding protein 45), that contains a region highly homologous to the nucleosome binding domain of the HMG-14/-17 proteins but is clearly distinct from these ubiquitous nuclear proteins. NBP-45 is a 406-amino acid-long protein. The N-terminal region of NBP-45 contains several domains that are homologous to evolutionarily conserved functional domains of the HMG-14/-17 protein family; however, most of the protein (85% of the sequence) has an unusually high content of negatively charged amino acids (42%). We demonstrate that NBP-45 is a nuclear protein, that it binds specifically to nucleosome core particles, that it is expressed in variable amounts in mouse tissues, and that NBP-45 transcripts are especially abundant in the RNA isolated from 7-day-old embryos. Our findings suggest that proteins other than HMG-14 or HMG-17 contain a functional nucleosomal binding domain (NBD) that may act as a module that facilitates the binding of nuclear proteins to chromatin. Thus, all the known functional motifs present in the ubiquitous HMG proteins are also found embedded in other, non-HMG nuclear proteins.

**MATERIALS AND METHODS**

**cDNA Cloning and Sequence Analysis**—The EST data base from several organisms was searched with the protein sequence EP-nucleosomal binding domain; EST, expressed sequence tag; GFP, green fluorescent protein.
KRSARLSA using the TBLASTN program (National Center for Biotechnology Information (NCBI)). A mouse EST clone (accession number AI046663) contained the above sequence but was clearly not an HMG-14 or an HMG-17 protein. The clone, in Escherichia coli DH10B, was obtained from Research Genetics. The plasmid DNA was purified by the alkaline lysis procedure using the EndoFree plasmid kit (Qiagen) and the insert excised by digestion with DraIII and XhoI. The insert was blunt ended with Klenow fragment and subcloned into vector pCR-BluntII TOPO (Invitrogen) and propagated in bacterial strain DH5a, and its sequence was determined with the Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems) and a 377 DNA sequencer (Perkin-Elmer Applied Biosystems).

Northern Hybridization—Mouse RNA master blot, mouse MTN blot, and mouse embryo MTN blots were obtained from CLONTECH (Palo Alto, CA) and probed, as recommended by the manufacturer, either with 32P-labeled the full-length cDNA or with a 32P-labeled fragment spanning nucleotides 43–418 of the cDNA. After hybridization, the membranes were washed with 0.1% SSC (15 mM NaCl, 1.5 mM sodium citrate), 0.1% SDS at 50 °C, and the radioactive signal was visualized with a PhosphorImager (Molecular Dynamics) and quantified using ImageQuant software (Molecular Dynamics).

Construction of Expression Plasmids in Mammalian and Bacterial Cells—cDNA fragments encoding amino acids 1–406, 24–406, or 1–108 were amplified with the polymerase chain reaction using Pfu DNA polymerase (Stratagene). All of the 5′-primers contained a BamHI site, whereas the 3′-primers for bacterial expression contained a HindIII site. The amplified DNA fragments were purified by agarose gel electrophoresis, extracted from the agarose gel by the GeneClean II kit (Biolab Inc), and digested with either BamHI/SalI or BamHI/HindIII. These fragments were ligated into either the BamHI/SalI site of the mammalian expression plasmid pCMV-4C (Stratagene), which codes for FLAG fusion proteins, or into the BglII/SalI site of the mammalian expression plasmid pEGFP-N2 (CLONTECH), which codes for green fluorescent protein (GFP)-tagged proteins. For bacterial expression, the DNA fragments were ligated into the BamHI/HindIII site of plasmid pET-23a (Novagen), which codes for T7-tagged fusion proteins. All the constructs were propagated in E. coli DH5a. The sequence of each of the insert was confirmed by sequence analysis.

Expression and Purification of Recombinant NBP-45 in Bacteria—All of the bacterial expression plasmids were transformed into E. coli BL21 (DE3). E. coli cells containing the expression construct were selected and grown in LB medium containing 100 µg/ml ampicillin, at 37 °C. Protein expression was induced when the A600 of the culture was 0.4–0.6 by adding isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.4 mM, and the cells were grown for an additional 3 h at 37 °C. The E. coli cells were harvested by centrifugation, suspended in 1/50 volume of BagBuster solution (Novagen), and then incubated for 10 min at room temperature with gentle shaking. The supernatant containing expressed proteins was separated by centrifugation at 10,000 × g for 30 min at 4 °C, and the T7-tagged proteins were purified on a T7 affinity resin (Novagen) followed by ion exchange chromatography on a Mono Q column, (Amersham Pharmacia Biotech) as recommended by the manufacturer. After elution from the resin, the solution containing recombinant protein was dialyzed against NEH buffer (10 mM Hepes-NaOH, pH 7.5, 10 mM NaCl, 1 mM EDTA). The protein concentration was determined with the BCA Protein assay kit (Pierce) using bovine serum albumin as a standard.

Transformation into Mammalian Cells and Detection of the Expressed Proteins by Western Blotting and Fluorescence Microscopy—HeLa cells grown in 3-cm-diameter dishes were transfected, using the LipoFe
toAMEX transfection reagent (Life Technologies, Inc.), with 0.7 µg of plasmid DNA expressing either the entire, or the truncated cDNA fragments, as recommended by the manufacturer. For Western analysis, 48 h after transfection the cells were harvested by centrifugation at 800 × g, 4 °C, for 10 min, the pellets were suspended in SDS gel loading buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 5% β-mercaptoethanol, 6% glycerol), and the cell lysates were electrophoresed in 15% SDS-polyacrylamide gel. The protein bands were transferred to Immobilon-P membranes (Millipore). After blocking in TBS-T (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) containing 10% dried milk, the membranes were incubated with anti-FLAG monoclonal antibody (Sigma, 0.8 µg/ml in TBS-T containing 10% dried milk) for 1 h at room temperature, washed, and reacted with anti-mouse IgG peroxidase conjugate (Pierce). The bound antibodies were detected with ECL Western blotting detection reagent (Amersham Pharmacia Biotech). For detection of GFP fusion protein, the transfected cells were grown on a coverslip, washed with phosphate-buffered saline twice, and observed under mi
croscopy using the appropriate filters. For detection of FLAG fused protein, cells grown on a coverslip were fixed with 2% formaldehyde in phosphate-buffered saline for 10 min at room temperature and then permeabilized with TNBS (0.1% Triton X-100, 1% bovine serum, 0.1% NaN3, in phosphate-buffered saline) for 20 min at room temperature. The location of the FLAG conjugates was visualized as described elsewhere (15), using anti-FLAG antibody (8 µg/ml in TNBS) as the first antibody and anti mouse IgG antibody conjugated with fluorescein isothiocyanate (Roche Molecular Biochemicals) as the second antibody.

RESULTS AND DISCUSSION

Cloning Strategy, Expression, and Characterization of NBP-45 Protein—The signature of the HMG-14/17 proteins is a stretch of 28 amino acids comprising the nucleosome binding domain of these nonhistone chromosomal proteins (11, 18). In the N-terminal half of this region, 10 out of 11 amino acid positions are invariant among all known members of the HMG-14/17 protein family. We used the sequence EPKRRSASRLL (GenBank™ accession number AI046663) that contained this invariant HMG-14/17 motif. The insert from clone AI046663 was obtained by digestion with DraIII and XhoI, and the resulting 1889-nucleotide-long DNA fragment was sequenced (Fig. 1A). Northern analysis of mouse liver RNA, with a DNA fragment corresponding to nucleotides 43–418, revealed a single band with an approximate molecular mass of 1.9 kilobases (Fig. 1B), suggesting that the sequenced fragment represents all or most of the single transcript present in this tissue.

The DNA contained an open reading frame encoding a 406-amino acid-long protein (Fig. 1A). Multiple sequence alignment revealed that this protein contained several regions that are homologous to structural domains characteristic of the HMG-14/17 protein family (bold letters in Fig. 1A and D). Thus, the first 4 amino acids, PRRK of this protein are also the first 4 amino acids in all the known HMG-14/17 proteins. The region spanning amino acids 13–40 of this protein is homologous to the highly conserved nucleosomal binding domain of the HMG-14/17 protein family, and the peptide AENGEAK, spanning amino acids 77 and 83, is homologous to the highly conserved peptide region in the C-terminal of all the HMG-14/17 proteins. Thus, the N-terminal portion of the new protein contains three regions that are identical or highly homologous to regions known to functionally relevant in HMG-14/17 proteins. The first region of homology is part of the bipartite nuclear localization signal of HMG-14/17 (19), the second region is their first region of homology is part of the bipartite nuclear localization signal of HMG-14/17 (19), the second region is their chromatin unfolding domain (21, 22).
FIG. 1. Cloning and expression of NBP-45. A, the cDNA and protein sequence of NBP-45. Bold letters in the protein sequence denote sequence identity with the HMG-14/-17 proteins. Acidic residues are marked in red (Glu) and blue (Asp). Overlapping repeated protein sequence motifs are indicated by colored lines.

B, a single mRNA codes for NBP-45; Northern analysis of poly(A) RNA extracted from mouse liver. A cloned DNA fragment spanning nucleotides 43–418 of the cDNA was used as a probe.

C, expression of NBP-45 protein. Lane 1, Western analysis, with anti T7-tag antibodies, of an SDS-polyacrylamide gel containing purified bacterially expressed T7-tagged NBP-45; lanes 2 and 3, Western analysis, using anti FLAG IgG, of affinity purified FLAG-binding protein from cellular extracts of HeLa cells transfected with either parent vector (lane 2) or with vector expressing the FLAG-labeled NBP-45 (lane 3).

D, sequence alignment of NBP-45 and mouse HMG-14/-17 proteins. Bold black
The calculated molecular mass of the protein is 45 kDa. Because the protein contains a putative nucleosomal binding domain it was named NBP-45 (nucleosomal binding protein 45).

To verify that the open reading frame indeed encodes a protein, we transfected HeLa cells with an expression plasmid containing a truncated cDNA sequence in which the FLAG tag was inserted at the TAA termination codon. Western analysis of the protein purified from the transfected cells revealed the presence of a single protein with an apparent molecular mass of 64 kDa (Fig. 1C, lanes 2 and 3). Likewise, bacterial expression of the entire open reading frame fused at the N-terminal with a T7 tag, produced a protein with the same molecular mass (Fig. 1C, lane 1). Apparently NBP-45 has an anomalous electrophoretic mobility in SDS-containing polyacrylamide gels, perhaps because of its unusual, highly acidic, amino acid composition (23, 37).

NBP-45 is a 406-amino acid-long protein and contains several overlapping repetitive sequence motifs (Fig. 1A). It is a highly acidic protein containing 110 glutamic acid and 44 aspartic acid residues, i.e. 37.9% of the residues are negatively charged. Only 16% of the residues are positively charged, and the protein has a calculated pI of 4.2. The charged residues are asymmetrically distributed along the polypeptide chain: in the first 50 N-terminal amino acids there are 13 positively charged residues and only three negatively charged residues, i.e. the basic to acidic ratio is 4, whereas in the rest of the protein this ratio is 0.34. The protein contains 7 arginine residues. Strikingly, 6 of these are found in the first 41 amino acids. NBP-45 has an unusually low content of aromatic amino acids, it contains two phenylalanines and one tyrosine and lacks tryptophan residues. The lack of tryptophan, the low content of aromatic residues, the clustering of arginine residues, and the asymmetric distribution of charged residues along the polypeptide chain are characteristic of the HMG-14/-17 protein family (11, 24). Thus, NBP-45 bears significant structural similarity to the nucleosomal binding proteins HMG-14/-17.

However, NBP-45 is clearly a new protein that is distinct from the HMG-14/-17 proteins. HMG-14 and HMG-17 are relatively small molecular mass proteins containing less than 100 amino acids. NBP-45 contains 406 amino acids; only the N-terminal 85 amino acids are homologous to HMG-14/-17 proteins. The rest of the protein is extremely acidic and has an unusually high content of glutamic acid residues, similar to that of a set of glutamic acid-rich proteins (GARPs) found in rod photoreceptors (25).

**NBP-45 Is a Nuclear Protein**—To test whether NPB-45 is indeed a nuclear protein, we transfected HeLa cells with plasmids expressing either NBP-45-GFP fusion constructs or NBP-45 containing the FLAG tag at its C-terminal. Fluorescence microscopy clearly indicated that both constructs efficiently localized to the nucleus (Fig. 2, A and B). Likewise, a FLAG-tagged NBP-45 deletion mutant lacking the 298 C-terminal amino acid residues did accumulate into the nuclei (Fig. 2C). In contrast, a deletion mutant of the NBP-45-GFP construct lacking the 23 N-terminal amino acid residues failed to enter the nucleus and accumulated in the cytoplasm (Fig. 2D), suggesting that this protein region contains at least one element necessary for nuclear entry. We already demonstrated that for HMG-14/-17, the first 4 amino acids PKRK, are part of the nuclear localization signal (19). Because this invariant sequence is also present in NBP-45, we suggest that these residues are part of its nuclear localization signal. We conclude that NPB-45 is a nuclear protein and that, similar to the nucleosomal binding proteins HMG-14/-17, its nuclear entry is mediated by an intrinsic nuclear localization signal.

**NBP-45 Binds Specifically to Nucleosome Cores**—The 147-base pair-long nucleosome core particle is the basic building block of the chromatin fiber. The only nuclear proteins known to recognize structural features of this chromatin subunit and bind to it specifically, independent of the underlying DNA sequence, are the ubiquitous HMG-14/-17 proteins. The main site of interaction between the HMG-14/-17 proteins and the nucleosome core particle is the highly conserved nucleosomal binding domain (20, 26, 27). Because a region of NBP-45 (amino acids 12–36; Fig. 1) is highly homologous to the nucleosomal binding domain of HMG-14/-17, we tested whether this protein also binds specifically to nucleosome core particles. To this end, we expressed the full-length NBP-45, an N-terminal deletion mutant lacking the first 23 N-terminal amino acids, and a 108-amino acid-long, C-terminal deletion mutant lacking the last 298 amino acid residues, in bacteria. All of the proteins were affinity purified using the T7 epitope, which was fused at the N-terminal (Fig. 3A).

Mobility shift assays of the full-length NBP-45 protein with 147-base pair-long DNA isolated from chicken core particles or with a mixture of this DNA and isolated nucleosome core particles clearly indicated that the protein binds specifically to the chromatin subunits. Thus, addition of increasing amounts of NBP-45 to 147-base pair DNA produced nonspecific smears and aggregates that accumulated at the top of the native polyacrylamide gels (Fig. 3B, lanes 1–5). In contrast, when added to a mixture of DNA and nucleosome core particles, the protein produced a specific band (Fig. 3B, lanes 6–10). The appearance of the specific mobility shift correlated with the depletion of the nucleosome core particle band. Significantly, although the

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*gray letters* indicate regions of full or partial similarity between NBP-45 and HMG-14/-17 proteins. The nucleosomal binding domain of HMG-14/-17 is indicated below the sequence.
Novel Nucleosomal Binding Protein

FIG. 3. The interaction of NBP-45 with nucleosome core particles. A, SDS-polyacrylamide gel electrophoresis and Western analysis of bacterially expressed NBP-45 (lane 1), a deletion mutant lacking the first 23 amino acids (lane 2), and a C-terminal deletion mutant (lane 3). All the proteins were T7-tag labeled. Left side, Coomassie Blue stain; right side, corresponding Western, developed with anti T7-tag antibodies. B, the addition of increasing amounts of nucleosome core-sized DNA produces nonspecific smearing (lanes 1–5), whereas the addition of the protein to a mixture of this DNA and core particles (CP) produces a specific mobility shift (CP+NBP, lanes 6–10). C, delineation of the nucleosomal binding domain of NBP-45. Addition of increasing amounts of either full-length NBP-45 (lanes 2–4) or its C-terminal deletion mutant (lanes 8–10) produce specific shifts with nucleosomes cores, whereas the N-terminal deletion mutant, lacking part of the HMG-14/-17-like nucleosomal binding domain, does not (lanes 5–7). D, mobility shift assay of nucleosome cores with either NBP-45 or a mixture of NBP-45 and HMG-17 in either 2× TBE (cooperative binding) or 0.5× TBE (noncooperative binding). In all these experiments the molar ratio of protein to core particles varied from 0.2 to 2.2. The asterisk in the right panel of D indicates the position of the putative heterodimer.

band corresponding to the core particle was totally depleted, the band corresponding to free DNA was almost intact (Fig. 3B, lane 10), a clear indication that the binding of NBP-45 to core particles is significantly stronger that its binding to protein-free DNA. We conclude therefore that NBP-45 binds specifically to nucleosome core particles.

The N-terminal deletion mutant of NBP-45, lacking residues that are highly homologous to the nucleosomal binding domain of HMG-14/17, does not produce specific mobility shifts with core particles (Fig. 3C, lanes 5–7). In contrast, a C-terminal deletion mutant lacking 75% of the amino acids but containing the regions homologous to the HMG-14/17 nucleosomal binding domain produces a specific mobility shift (Fig. 3C, lanes 8–10). We conclude therefore that the main nucleosomal binding domain of NBP-45 is the region that is homologous to the nucleosomal binding domain of the ubiquitous HMG-14/17 proteins. We note, however, that the band produced by the C-terminal deletion mutant is somewhat diffuse and less well defined than that produced by the intact NBP-45 (Fig. 3C, lane 4, and B–D) or by the HMG-17 protein (Fig. 3D). Amino acid residues absent from the C-terminal deletion mutant may be necessary for stabilizing the interaction of NBP-45 with core particles.

The interaction of HMG-14/17 proteins with core particles is dependent on ionic strength. In 2× TBE, an ionic strength close to physiological, the proteins bind to the nucleosome core cooperatively and form homodimeric complexes containing either two molecules of HMG-14 or two molecules of HMG-17. At low ionic strength, the binding is noncooperative producing heterodimeric complexes containing one molecule of each HMG protein (28). We tested whether the interaction of NBP-45 with core particles is similar to that of the HMG-14/17 proteins.

In 2× TBE solution, the addition of core particles to a solution containing both HMG-17 and NBP-45 proteins produced only two type of nucleosome core complexes: one with a mobility characteristic of a homodimer of HMG-17 and one with a mobility characteristic of the NBP-45:core particle complex (Fig. 3D). Quantitative two-dimensional gel analysis suggests that this complex also contains two molecules of NBP-45. Under these conditions, the dissociation constant for the binding of NBP-45 to nucleosome cores, determined as described before (29), is $4.0 \times 10^{-7} \times \text{m}^{-1}$, a value similar to that of HMG-14/17 (29).

At low ionic strength, in 0.5× TBE solutions, the binding of NBP-45 to core particles still produces only one major complex, whereas the binding of HMG-17 is clearly noncooperative and produces complexes containing either one or two molecules of HMG-17 (Fig. 3D, lane 5). At this ionic strength, a mixture containing both HMG-17 and NBP-45 produces an additional band with a mobility intermediate between that of an NBP-45 homodimer and that of an HMG-17 homodimer, suggesting the existence of a heterodimeric complex containing one molecule of HMG-17 and one of NBP-45 (asterisk in Fig. 3D). The amount of protein in this band was too low for reliable quantification by two-dimensional gel electrophoresis.

We conclude that the interaction of NBP-45 with core particles and DNA is very similar to that of the ubiquitous HMG-14/17 protein. NBP-45 produces nonspecific complexes with DNA, binds specifically to nucleosome cores, and at physiological ionic strength produces homodimeric mixtures. Thus, NBP-45 is indeed a nucleosomal binding domain protein. This study is the first to experimentally demonstrate the existence of a functional HMG-14/17 nucleosomal binding domain in proteins other than the "canonical" HMG-14/17 protein themselves.

Delineation of the Main Functional Domains of NBP-45—The asymmetric distribution of charged residues along the NBP-45 polypeptide chain creates an unusually long protein domain of very high negative charge density. In the region
spanning residues 50–406 (85% of the protein) there are 108 glutamic acid and 41 aspartic acid residues, i.e. 42% of the residues are negatively charged. In numerous transcription factors, a negatively charged region is the protein domain mainly responsible for the transcription activation activity (30–33). To test the potential transcriptional activation activity of NBP-45, we cotransfected into HeLa cells, the reporter plasmid pCH110, in which the expression β-galactosidase is driven by the SV40 promoter, with plasmids expressing either intact or with C-terminal deletion mutants of FLAG tagged NBP-45. Western analysis of extracts from the transfected cells indicated that all of the polypeptides were expressed (Fig. 4A).

Cells transfected with full-length NBP-45 expressed 8-fold more β-galactosidase than cells transfected with the control vector pCMV-Tag4C (Fig. 4B). Deletion of the 214 C-terminal amino acids decreased the stimulation activity of NBP-45 from 8- to 4-fold. A longer deletion mutant lacking all but the first 108 amino acids stimulated transcription only 2-fold, i.e. 4-fold lower than the intact protein. The C terminus of this deletion mutant is still negatively charged; in the 42 C-terminal residues there are 17 negatively charged and only three positively charged amino acids. We conclude, therefore, that NBP-45 has the potential to function as a general transcriptional activator and that the transcriptional stimulation correlated with the length of the C-terminal region of the protein. The negatively charged residues may serve to mobilize additional transcription factors to form multiprotein complexes, as has been suggested for numerous site specific transcription activators, (30–33). Alternatively, by analogy to HMG-14/-17 proteins, the negatively charged domain may enhance transcription by modifying the higher order chromatin structure of the transfected plasmid (21, 22, 24–36).

The main functional domains of NBP-45 are outlined in Fig. 4C. The first 23 amino acids contain a region necessary for nuclear entry of the protein. The region spanning residues 12–40 is the main nucleosomal binding domain of the protein because it is highly homologous to the nucleosomal binding domain of HMG-14/-17, and the deletion of a region containing part of this region is sufficient to abolish nucleosomal binding. The negatively charged C-terminal domain activates transcription. The functional domains of NBP-45 are indicative of a nuclear protein that binds to nucleosomes, thereby creating a chromatin region of extremely high local negative charge.

### Tissue-specific and Developmentally Specific Expression of NBP-45

To gain insights into the tissue specificity and expression levels of NBP-45 we used the entire 1889-nucleotide-long cDNA sequence to query several data bases using the BLASTN (NCBI) program. In the data base of Expressed Sequence Tags, we obtained 15 hits of partial homology with mouse sequences expressed in blastocysts, T-cells, liver, hippocampus, and embryos. The non redundant data base (NCBI) contained a mouse sequence (GARP45, GenBank™ accession number AB018374) that was deposited while this work was in
progress, identical to NBP-45. We obtained three hits with rat EST clones and two hits with human EST clones. A similar analysis with the mouse HMG-14 or HMG-17 sequence yielded over 100 sequences that were identical to the query sequences. These findings suggest that the NBP-45 mRNA has a very low abundance or alternatively that the message is expressed in a tissue-specific manner.

We used a RNA Master Blot containing mRNA from 22 different mouse tissues (CLONTECH) to examine the tissue specific expression of NBP-45 transcripts. In this blot the amounts of RNA spotted are normalized to the transcription levels of eight housekeeping genes; therefore, the intensity of the signal is indicative of the relative mRNA abundance in a tissue. Quantitative analysis of the signal indicated that in adult mouse the abundance of the NBP-45 mRNA varied over a 6-fold range (Fig. 5, A and B). It was highest in the submaxillary gland, thymus, kidney, and liver and lowest in brain, lung, pancreas, and eye. Because Northern analysis with the same probe indicated that the mouse liver contains only a single size mRNA (Fig. 1B), it is highly likely that the signal is due only to the presence of NBP-45 mRNA. We conclude that NBP-45 mRNA is present in variable amounts in most and perhaps all adult mouse tissues.

NBP-45 mRNA was especially abundant in RNA extracted from 7-day-old embryos. At this developmental stage the abundance of NBP-45 is 4-fold higher than that of any adult tissue and almost 10-fold higher than that present in the uterus, ovary, or later embryonic stages (11, 15, 17-day-old embryos). Northern analysis of RNA obtained at the different embryonic stages verified that the probe hybridizes to a single, 1.9-kilobase mRNA species and that the abundance of this species was highest in the RNA isolated from 7-day-old embryos (Fig. 5C).

Taken together, the results suggest that NBP-45 is expressed at low levels and in variable amounts in most and perhaps all adult mouse tissues. The presence of NBP-45 transcripts in all the mouse tissues tested suggest that this protein may have a housekeeping function. We postulate that the binding of NBP-45 to nucleosomes would introduce a high local density of negative charges, which could lead to significant structural changes in chromatin. In addition, the elevated levels of transcripts in 7-day-old embryos suggest that the protein may have an important function during specific developmental stages.

A New Protein Motif: The Nucleosome Binding Domain—The only protein domain known to bind preferentially to the 147-base pair nucleosome core particle in a sequence-independent fashion is the NBD, which until now was functionally detected only in the canonical HMG-14/17 nuclear proteins. A possible protein closely related to the HMG-14/17 proteins, Trip-7, had its NBD, which until now was functionally detected only in the canonical HMG-14/17 nuclear proteins and has not been isolated, and its interaction with nucleosomes has not been examined. We now report the cloning and characterization of a new protein, which we named NBP-45 that contains a functional NBD.

NBP-45 is clearly a new type of protein, which is significantly different from the ubiquitous HMG-14/17 proteins. These findings suggest that the NBD protein motif may be more widespread than previously thought and that the NBD may be embedded in nuclear proteins that target the nucleosomal chromatin structure. In this respect, the nucleosomal binding domain, i.e. the functional motif of the HMG-14/17 protein family, resembles the functional motifs of the two other members of the HMG proteins: the HMG-1/2 and the HMG-I/Y/C proteins. The functional motifs of these proteins, the HMG-1 box and the AT-hook, have been identified in numerous nuclear proteins that interact with DNA (10–13). Thus, all of the functional motifs present in the ubiquitous, evolutionarily conserved HMG proteins are also present in non-HMG proteins. A characteristic property of all the HMG functional motifs is their ability to modify the structure of their binding site and induce a conformation that facilitates the progression of DNA-dependent activities such as transcription and replication (10). Our studies establish the NBD as a general protein motif, which is found in other than just HMG-14/17 proteins. This protein motif may facilitate binding to nucleosomes in chromatin.

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J. Biol. Chem. 2000, 275:6368-6374.
doi: 10.1074/jbc.275.9.6368

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